

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 November 2007 (08.11.2007)

PCT

(10) International Publication Number
WO 2007/126402 A2

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2006/004920

(22) International Filing Date:
13 February 2006 (13.02.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/652,177 11 February 2005 (11.02.2005) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

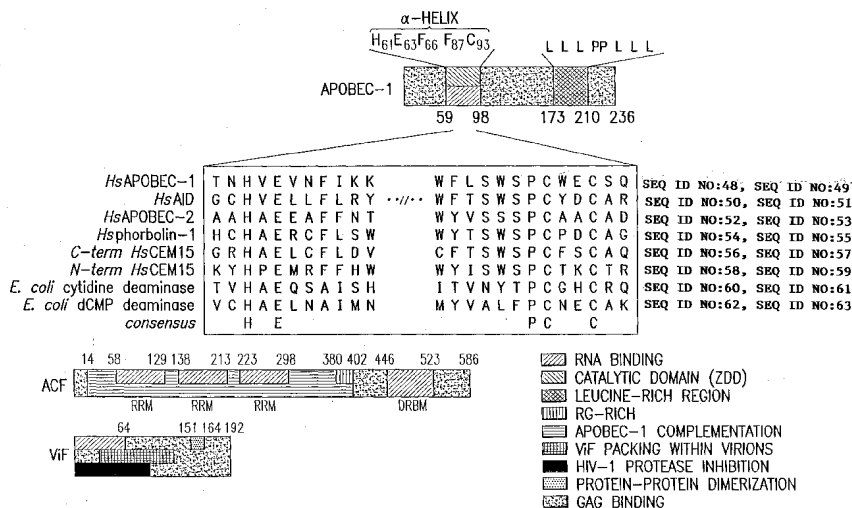
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished upon receipt of that report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau
- the filing date of the international application is within two months from the date of expiration of the priority period

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS AND COMPOSITIONS RELATED TO HEIGHTENED APOBEC-1 RELATED PROTEIN (ARP) EXPRESSION



(57) Abstract: Disclosed are methods and compositions related to determining the status and mechanisms underlying long-term nonprogression of viral infections.

**METHODS AND COMPOSITIONS RELATED TO HEIGHTENED APOBEC-1
RELATED PROTEIN (ARP) EXPRESSION**

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No. 60/652,177, filed
5 February 11, 2005, which is hereby incorporated herein by reference in its entirety.

ACKNOWLEDGEMENTS

This invention was made with support under grants R01-AI05163, U01-AI27658, and
R21 58789-01 from the National Institutes of Health and grant F49620-01 from the Air Force.
10 The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Human white blood cells express proteins called APOBEC-1 related proteins (ARPs),
which are cytidine deaminases that can change the genetic code of an infecting virus. These
15 changes can render the virus incapable of producing an infection when they occur in critical
genes encoding viral proteins and/or when they occur extensively throughout the viral genome.
APOBEC-1 related proteins (ARPs), such as CEM-15, APOBEC-3B, APOBEC-3C, and
APOBEC-3F have been found to have a deleterious effect on HIV-1, HIV-2, retrovirus and
hepatitis B. HIV-1, however, expresses a protein called Viral infectivity factor (Vif) that impairs
20 the ability of ARPs such as CEM15 to act on viral DNA.

A small subset of HIV-infected individuals, known as long-term nonprogressors
(LNTPs) have substantially slower rates of disease progression in the absence of therapeutic
intervention. Clinically, these LTNP are usually asymptomatic, maintain high CD4 counts and
low HIV viremia levels. The characteristics are therefore of prognostic value in evaluating
25 disease severity. The mechanisms responsible for long-term nonprogression have been
attributed to defective or less fit HIV variants, strong host immune responses and unique host
genetic elements, such as the CCR5 genotype and HLA haplotypes (Buchbinder et al. (1999)
Microbes and Infection 1:1113-1120).

Thus, needed in the art are methods and compositions related to determining the status
30 and mechanisms underlying long-term nonprogression of viral infections. More specifically, the
role of APOBEC-1 related proteins in viral progression and its affect in long-term
nonprogressors is of importance.

SUMMARY OF THE INVENTION

In accordance with the purposes of this invention, as embodied and broadly described herein, this invention, in one aspect, relates to a method of predicting the severity of a viral infection in a subject. For example, the level of expression of at least one APOBEC-1 related protein is used to indicate the level of severity.

Also disclosed is a method of predicting whether a subject is or will be a long term nonprogressor (LTNP) when infected with a virus. A higher level of expression in a biological sample from the subject of one or more APOBEC-1 related proteins as compared to a control level indicates the subject is a potential LNTP.

Further disclosed is a method of optimizing antiviral therapy in a subject with a viral infection. The level of expression of one or more APOBEC-1 related proteins (ARPs) in a biological sample from the subject is used to adjust the antiviral therapy, thereby optimizing the viral therapy.

Also disclosed is a method of predicting the level of CD4 cells in a subject. The level of CEM15 correlates with the level of CD4 cells and can be used to predict the level of CD4 cells.

Also disclosed is a method of monitoring effectiveness of an antiviral agent in a subject. Specifically, expression levels of one or more APOBEC-1 related proteins are monitored during the treatment. An increase in expression levels of the APOBEC-1 related proteins during the course of treatment indicates the effectiveness of the antiviral agent.

Further disclosed is a method of screening for an antiviral agents and compositions used to detect levels of ARP expression, including nucleic acid primers and probes.

Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principles of the invention.

Figure 1 shows representative members of the APOBEC-1 related family of cytidine deaminases including CEM15. Also shown are APOBEC-1 complementation factor (ACF) and

viral infectivity factor (Vif). The catalytic domain of APOBEC-1 is characterized by a ZDD with three zinc ligands (either His or Cys), a glutamic acid, a proline residue and a conserved primary sequence spacing (Mian, I.S., *et al.*, (1998) J Comput Biol. 5:57-72.). The ZDD of other deaminases and APOBEC-1 related proteins is shown for comparison along with a consensus ZDD. The indicated residues in the catalytic site of APOBEC-1 bind AU-rich RNA with weak affinity. The leucine rich region (LRR) of APOBEC-1 has been implicated in APOBEC-1 dimerization and shown to be required for editing (Lau, P.P., *et al.*, (1994) Proc Natl Acad Sci U S A. 91:8522-6; Oka, K., *et al.*, (1997) J Biol Chem. 272:1456-60.) but structural modeling suggests that LRR forms the hydrophobic core of the protein monomer (Navaratnam, N., *et al.*, (1998) J Mol Biol. 275:695-714.). ACF complements APOBEC-1 through its APOBEC-1 and RNA binding activities. The RNA recognition motifs (RRM)s are required for mooring sequence-specific RNA binding and these domains plus sequence flanking them are required for APOBEC-1 interaction and complementation (Blanc, V., *et al.*, (2001) J Biol Chem. 276:46386-93.; Mehta, A., *et al.*, (2002) RNA. 8:69-82.) APOBEC-1 complementation activity minimally depends on ACF binding to both APOBEC-1 and mooring sequence RNA. A broad APOBEC-1 complementation region is indicated that is inclusive of all regions implicated in this activity (Blanc, V., *et al.*, (2001) J Biol Chem. 276:46386-93.; Mehta, A., *et al.*, (2002) RNA. 8:69-82.) Experiments have shown the N-terminal half of Vif is necessary for viral infectivity (Henzler, T. 2001). However, reports have demonstrated that residues in the C-terminus (amino acids 151-164) are essential for infectivity (Yang, S. *et al.* 2001) and that multimerization of Vif through the motif PPLP (SEQ ID NO: 14) within this region was essential for infectivity. Peptides capable of binding to this domain of Vif blocked Vif-Vif interactions and Vif-Hck interactions *in vitro* and suppressed viral infectivity in cell-based assay systems. Residues in the N-terminus of Vif are essential for RNA binding and packing of Vif within the virion (Zhang *et al.* 2000; Khan *et al.* 2001; Lake *et al.* 2003).

Figure 2 shows schematic depictions of the cytidine deaminase (CDA) polypeptide fold and structure-based alignments of APOBEC-1 with respect to its related proteins (ARPs). Figure 2a depicts a gene duplication model for cytidine deaminases. CDD1 belongs to the tetrameric class of cytidine deaminases with a quaternary fold nearly identical to that of the tetrameric cytidine deaminase from *B. subtilis* (Johansson, E., *et al.*, (2002) Biochemistry. 41:2563-70.). Such tetrameric enzymes exhibit the classical $\alpha\beta\alpha\beta\alpha\beta\beta$ topology of the Zinc Dependent Deaminase Domain (ZDD) observed first in the Catalytic Domain (CD) of the dimeric enzyme from *E. coli* (Betts, L., *et al.*, (1994) J Mol Biol. 235:635-56). According to the gene duplication model, an ancestral CDD1-like monomer (upper left ribbon) duplicated and

fused to produce a bipartite monomer. Over time a C-terminal Pseudo-Catalytic Domain (PCD) arose that lost substrate and Zn^{2+} binding abilities (upper right ribbon). The model holds that the interdomain CD-PCD junction is joined via flexible linker that features conserved Gly residues necessary for catalytic activity on large polymeric DNA or RNA substrates. The function of the PCD is to stabilize the hydrophobic monomer core and to engage in auxiliary factor binding. The loss of PCD helix $\alpha 1$ can provide a hydrophobic surface where auxiliary factors bind to facilitate substrate recognition thereby regulating catalysis. The enzymes remain oligomeric because each active site comprises multiple polypeptide chains. Modern representatives of the chimeric CDA fold include the enzyme from *E. coli*, as well as APOBEC-1 and AID. Other ARPs such as APOBEC-3G (CEM15) may have arisen through a second gene duplication to produce a pseudo-homodimer on a single polypeptide chain (lower ribbon); structural properties of the connector polypeptide are unknown. Signature sequences compiled from strict structure-based alignments (upper) are shown below respective ribbon diagrams, where X represents any amino acid. Linker regions (lines) and the location of Zn^{2+} binding (spheres) are depicted. Although experimental evidence suggests APOBEC-3B has reduced Zn^{2+} binding and exists as a dimer (Jarmuz, A., *et al.*, (2002) *Genomics*, 79:285-96), modeling studies suggest it will bind Zn^{2+} (as shown in Wedekind *et al. Trends Genet*, 19(4):207-16, 2003) and may function as a monomer. Inset spheres represent proper (222) CDD1-like quaternary structure symmetry whereas APOBEC-1-like enzymes exhibit pseudo-222 symmetry relating CD and PCD subunits; in the latter enzyme a proper dyad axis relates the polypeptide chains. Finally, APOBEC-3G can fold as a monomer from a single polypeptide chain with each CD and PCD (differently colored spheres in lower left inset box) related by improper 222 symmetry with no strict axes of symmetry. Figure 2b depicts the structure based sequence alignment for ARPs. Sequences from human APOBEC-1, AID, and APOBEC-3G were aligned based upon a main-chain alpha-carbon least-squares superposition of the known cytidine deaminase three dimensional crystal structures from *E. coli*, *B. subtilis* and *S. cerevisiae* (Figure 2c). Amino acid sequence alignments were optimized to minimize gaps in major secondary structure elements, which are depicted as tubes (α -helices) and arrows (β -strands) in Figure 2b. Additionally, loops, turns, and insertions of Figure 2b are marked L and T and i, respectively. L-C1 and L-C2 represent distinct loop structures in the dimeric versus tetrameric cytidine deaminases. Sections of basic residues that overlap the bipartite NLS of APOBEC-1 are marked BP-1 and BP-2. Figure 2d depicts a schematic diagram of the domain structure observed in APOBEC-1 and related ARPs based upon computer-based sequence alignments using the ZDD signature sequence shown in the lower panel of Figure 2a.

Figure 3 shows the relation of CEM15 amino acid sequence to APOBEC-1 and other APOBEC-1 Related Proteins (ARPs) by use of standard computational methods based upon amino acid similarity or identity. Amino acid sequence alignments illustrate conservation of Zn^{2+} ligands and key catalytic residues essential to the mechanism of hydrolytic deamination by cytidine deaminases (CDA). Collectively, these amino acids form a signature zinc-dependent deaminase domain (ZDD), present in: (i) APOBEC-1, which mediates C to U editing of apoB mRNA, (ii) the Activation Induced Deaminase (AID), which mediates Somatic Hypermutation (SHM) and Class Switch Recombination (CSR), and (iii) CEM15, which blocks HIV-1 viral infectivity.

Figure 4 shows reduced production of pseudotyped HIV-1 viral particles by cells expressing CEM15 or DM. p24 concentration (pg/ml) normalized to % GFP containing cells (as a measure of transfection efficiency) for 293T cells stably expressing pIRES-P vector (n=6), CEM15 (n=6) and DM (n=5), following transfection with wild-type (Vif+) or Δ Vif proviral DNA plasmids (black and white bars, respectively). Error bars represent standard deviation calculated from n for each cell line.

Figure 5 shows CEM15 suppresses HIV-1 protein abundance. 293T cell lines stably expressing (A) CEM15, (B) DM, and (C) control pIRES-P vector were transiently transfected with proviral HIV-1 plasmids (containing either wild-type Vif (+) or Δ Vif (-)). Total cell lysates were prepared at 24, 48, and 72 hours post-transfection, separated by SDS-PAGE and analyzed by immunoblot assay using antibodies reactive with HA (HA-tagged CEM15 and DM), Vif, p24, RT, β -actin, Vpr, or Tat (as denoted on the left). The molecular weight (kDa) of the indicated protein species is given to the right.

Figure 6 shows CEM15 suppresses HIV-1 viral RNA abundance. (A) Location of Gag-Pol junction and protease region of HIV-1 genomic RNA corresponding to the GP-RNA probe used for RNA binding and Northern blot analysis. (B) UV crosslinking of increasing concentration of recombinant CEM15 protein (1, 2 and 4 μ g protein) to 20 fmol radiolabeled GP-RNA and apoB RNA. (C) Poly A+ RNA abundance for Gag-Pol transcripts in 293T-CEM15 at 24, 48, and 72 hours and DM cells at 48 hours post-transfection with Vif+ (black) and Δ Vif (white) proviral DNA. Results are expressed as the ratio of viral RNA (GP-RNA region) to endogenous cellular RNA (adenovirus E1A) determined through phosphorimager scanning densitometry analysis of Northern blots.

Figure 7 shows real-time PCR assay for CEM15 gene expression. Samples of polyA+ mRNA were amplified from a positive control (CEM15 plasmid patient samples (patient #7 is shown) and internal control of GAPDH (inset) from reverse transcribed cDNA using the real-

time PCR as described in Example 1. Results showed linear amplification of CEM15 and GAPDH mRNAs from human PBMC. Using this assay, CEM15 and GAPDH mRNAs were quantified in each patient sample (Table 3).

Figure 8 shows the protective effects of increased CEM15 gene expression. Single linear regression analysis between GAPDH normalized CEM15 mRNA levels and HIV viremia or CD4 counts in eight HIV-infected individuals. Results showed a strong inversed correlation between CEM15 gene expression and viremia levels (a), and a significant positive correlation between CEM15 gene expression and CD4 counts (b).

DETAILED DESCRIPTION

The APOBEC-1 and APOBEC-1 related compositions described herein are useful in preventing or treating viral infections. Described herein are methods of identifying long term nonprogressors, optimizing antiviral infectivity therapy, predicting the severity of a viral infection in a subject, predicting the level of CD4 cells in a subject, monitoring the effectiveness of an antiviral agent, and screening for an antiviral agent. Also disclosed are nucleic acid sequences used to detect expression of ARPs.

The present invention may be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the Examples included therein and to the Figures and their previous and following description.

Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that this invention is not limited to specific synthetic methods, specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

As used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a pharmaceutical carrier” includes mixtures of two or more such carriers, and the like.

Ranges may be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of

each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as “about” that particular value in addition to the value itself. For example, if the value “10” is disclosed, then “about 10” is also disclosed. It is also understood that when a value is disclosed that “less than or equal to” the value, “greater than or equal to the value” and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value “10” is disclosed the “less than or equal to 10” as well as “greater than or equal to 10” is also disclosed.

In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

“Optional” or “optionally” means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

The terms “higher,” “increases,” “elevates,” “enhances,” or “elevation” refer to increases as compared to a control. The terms “low,” “lower,” “reduces,” “suppresses” or “reduction” refer to decreases as compared to a control level. Control levels can be normal *in vivo* levels prior to, or in the absence of, an infection or a treatment. Thus, the control can be from the same subject prior to infection or treatment or can be an uninfected or untreated control subject or group thereof.

The term “test compound” is defined as any compound to be tested for its ability to bind to increase ARP activity, production, or expression. “Test compounds” include drugs, molecules, and compounds that come from combinatorial libraries where thousands of such ligands are screened by drug class.

By “subject” is meant an individual. Preferably, the subject is a mammal such as a primate, and, more preferably, a human. The term “subject” can include domesticated animals, such as cats, dogs, etc., livestock (e.g., cattle, horses, pigs, sheep, goats, etc.), and laboratory animals (e.g., mouse, rabbit, rat, guinea pig, etc.).

The terms “control levels” or “control cells” are defined as the standard by which a change is measured, for example, the controls are not subjected to the experiment, but are instead subjected to a defined set of parameters, or the controls are based on pre- or post-treatment levels.

By “contacting” is meant an instance of exposure of at least one substance to another substance. For example, contacting can include contacting a substance, such as a cell, or cell to a test compound described herein. A cell can be contacted with the test compound, for example,

by adding the protein or small molecule to the culture medium (by continuous infusion, by bolus delivery, or by changing the medium to a medium that contains the agent) or by adding the agent to the extracellular fluid *in vivo* (by local delivery, systemic delivery, intravenous injection, bolus delivery, or continuous infusion). The duration of contact with a cell or group of cells is determined by the time the test compound is present at physiologically effective levels or at presumed physiologically effective levels in the medium or extracellular fluid bathing the cell. In the present invention, for example, a virally infected cell (e.g., a, HIV infected cell) or a cell at risk for viral infection (e.g., before, at about the same time, or shortly after HIV infection of the cell) is contacted with a test compound.

“Treatment” or “treating” means to administer a composition to a subject or a system with an undesired condition or at risk for the condition. The condition can be any pathogenic disease, autoimmune disease, cancer or inflammatory condition. The effect of the administration of the composition to the subject can have the effect of but is not limited to reducing the symptoms of the condition, a reduction in the severity of the condition, or the complete ablation of the condition.

By “effective amount” is meant a therapeutic amount needed to achieve the desired result or results, e.g., reducing viral infectivity, blunting physiological functions, altering the qualitative or quantitative nature of the proteins expressed by cell or tissues, and eliminating or reducing disease causing molecules and/or the mRNA or DNA that encodes them, etc.

Herein, “inhibition” or “suppression” means to reduce activity as compared to a control (e.g., activity in the absence of such inhibition). It is understood that inhibition or suppression can mean a slight reduction in activity to the complete ablation of all activity. An “inhibitor” or “suppressor” can be anything that reduces the targeted activity.

“Suppression of viral activity” is defined as a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 2-fold, 10-fold, 100-fold, or 1000-fold suppression of viral activity. Viral activity includes, but is not limited to, viral reproduction, viral shedding, or viral infectivity.

Many methods disclosed herein refer to “systems.” It is understood that systems can be, for example, cells, columns, or batch processing containers (e.g., culture plates). A system is a set of components, any set of components that allows for the steps of the method to performed.

Typically a system will comprise one or more components, such as a protein(s) or reagent(s). One type of system disclosed would be a cell that comprises both Vif and a test compound, for example. Another type of system would be one that comprises a cell and an infective unit (e.g., an HIV unit). A third type of system might be a chromatography column that has CEM15 or other ARPs bound to the column.

By “virally infected mammalian cell system” or “virally infected” is meant an *in vitro* or *in vivo* system infected by a virus. Such a system can include mammalian cellular components; mammalian cells, tissues, or organs; and whole animal systems. By “HIV infectivity” or “viral infectivity” is meant the capacity of an *in vitro* or *in vivo* system to become infected by an virus (e.g., an HIV virus).

By “Vif antagonist” is meant any molecule or composition that counteracts, reduces, suppresses, inhibits, blocks, or hinders the activity of a Vif molecule or a fragment thereof. This includes Vif dimerization antagonists, which reduce, suppress, inhibit, block, or hinder the dimerization of Vif. Any time a “Vif antagonist” is mentioned, this includes Vif dimerization antagonists. Also included are agents that block Vif binding to the CEM15, agents that block Vif-mediated polyubiquitination of CEM15, and the like.

By “cytidine deaminase activator” is meant any molecule or composition that enhances or increases the activity of a cytidine deaminase molecule or a fragment thereof. By cytidine deaminase activator is also meant deoxycytidine deaminase activator, ARP activator, or any related molecule.

By “deoxycytidine deaminase activator” is meant any molecule or composition that enhances or increases the activity of a deoxycytidine deaminase molecule or a fragment thereof.

By “ARP activator” is meant any molecule or composition that enhances or increases the activity of an APOBEC-1 Related Protein molecule or a fragment thereof.

A “cytidine deaminase-positive cell” means any cell that expresses one or more cytidine deaminases or deoxycytidine deaminases. Such expression can be naturally occurring or the cell can include an exogenous nucleic acid that encodes one or more selected deaminases.

“Primers” are a subset of probes that are capable of supporting some type of enzymatic manipulation and that can hybridize with a target nucleic acid such that the enzymatic manipulation can occur. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art which do not interfere with the enzymatic manipulation.

There are several examples of cellular and viral mRNA editing in mammalian cells. (Grosjean and Benne (1998); Smith et al. (1997) RNA 3: 1105-23). Two examples of such editing mechanisms are the adenosine to inosine and cytidine to uridine conversions. (Grosjean and Benne (1998); Smith et al. (1996) Trends in Genetics 12:418-24; Krough et al. (1994) J. Mol. Biol. 235:1501-31). Editing can also occur on both RNA and on DNA, and typically these functions are performed by different types of deaminases.

A to I editing involves a family of adenosine deaminases active on RNA (ADARs). ADARs typically have two or more double stranded RNA binding motifs (DRBM) in addition to a catalytic domain whose tertiary structure positions a histidine and two cysteines for zinc ion coordination and a glutamic acid residue as a proton donor. The catalytic domain is conserved at the level of secondary and tertiary structure among ADARs, cytidine nucleoside/nucleotide deaminases and CDARs but differs markedly from that found in adenosine nucleoside/nucleotide deaminases (Higuchi et al (1993) Cell 75:1361-70). ADAR editing sites are found predominantly in exons and are characterized by RNA secondary structure encompassing the adenosine(s) to be edited. In human exon A to I editing, RNA secondary structure is formed between the exon and a 3' proximal sequence with the downstream intron (Grosjean and Benne (1998); Smith et al. (1997) RNA 3: 1105-23; Smith et al. (1996) Trends in Genetics 12:418-24; Maas et al (1996) J. Biol. Chem. 271:12221-26; Reuter et al. (1999) Nature 399:75-80; O'Connell (1997) Current Biol. 7:R437-38). Consequently, A to I editing occurs prior to pre-mRNA splicing in the nucleus. The resultant inosine base pairs with cytosine and codons that have been edited, effectively have an A to G change. ADAR mRNA substrates frequently contain multiple A to I editing sites and each site is selectively edited by an ADAR, such as ADAR1 or ADAR2. ADARs typically function autonomously in editing mRNAs. ADARs bind secondary structure at the editing site through their double stranded RNA binding motifs or DRBMs and perform hydrolytic deamination of adenosine through their catalytic domain.

One example of a Cytosine Deaminase Active on RNA (CDAR) is APOBEC-1 (apolipoprotein B mRNA editing catalytic subunit 1) (accession # NM_005889) encoded on human chromosome 12. (Grosjean and Benne (1998); Lau et al. (1994) PNAS 91:8522-26; Teng et al (1993) Science 260:1816-19). APOBEC-1 edits apoB mRNA primarily at nucleotide 6666 (C6666) and to a lesser extent at C8702 (Powell et al. (1987) Cell 50:831-40; Chen et al. (1987) Science 238: 363-366; Smith (1993) Seminars in Cell Biology 4:267-78) in a zinc dependent fashion (Smith et al. (1997) RNA 3:1105-1123). This editing creates an in-frame translation stop codon, UAA, from a glutamine codon, CAA at position C6666 (Grosjean and Benne (1998); Powell et al. (1987) Cell 50:831-840; Chen et al. (1987) Science 238:363-66). The biomedical significance of apoB mRNA editing is that it results in increased production and secretion of B48 containing very low density lipoproteins and correspondingly, a decrease in the abundance of the atherogenic apoB100 containing low density lipoproteins in serum (Davidson et al. (1988) JBC 262:13482-85; Baum et al. (1990) JBC 265:19263-70; Wu et al. (1990) JBC 265:12312-12316; Harris and Smith (1992) Biochem. Biophys. Res. Commun. 183:899-903;

Inui et al. (1994) J. Lipid Res. 35:1477-89; Funahashi et al (1995) J. Lipid Res. 36:414-428; Giannoni et al. J. Lipid Res. 36:1664-75; Lau et al. (1995) J. Lipid Res. 36: 2069-78; Phung et al. (1996) Metabolism 45:1056-58; Van Mater et al. (1998) Biochem. Biophys. Res. Commun. 252:334-39; von Wronski et al. (1998) Metab. Clin. Exp. 7:869-73).

5 Activation induced deaminase, AID (GenBank accession # BC006296) is encoded on human chromosome 12 (Muto, 2000); (Muramatsu et al. (1999) JBC 274: 18740-76; Muramatsu et al. (2000) Cell 102:553-64; Revy et al. (2000) Cell 102:565-76). AID contains a ZDD (Zinc-dependent deaminase domain) and has 34% amino acid identity to APOBEC-1 (Table 3, Figure 5 and 6). Its location on human chromosome 12p13 suggests it may be related to APOBEC-1 by
10 a gene duplication event (Lau, 1994; Muto, 2000). This chromosomal region has been implicated in the autosomal recessive form of Hyper-IgM syndrome (HIGM2) (Revy, 2000). Most patients with this disorder have homozygous point mutations or deletions in three of the five coding exons, leading to missense or nonsense mutations (Revy, P., 2000) Cell. 102:565-75). Significantly, some patients had missense mutations for key amino acids within AID's ZDD
15 (Revy, 2000; Minegishi, 2000). AID homologous knockout mice demonstrated that AID expression was the rate limiting step for class switch recombination (CSR) and required for an appropriate level of somatic hypermutation SHM (Muramatsu, 2000). The expression of AID controls antibody diversity through multiple gene rearrangements involving mutation of DNA sequence and recombination.

20 Human APOBEC-2 (Genbank Accession # XM004087) is encoded on chromosome 6 and is expressed uniquely in cardiac and skeletal muscle (Liao et al. Biochem Biophys. Res. Commun. 260:398-404). It shares homology with APOBEC-1's catalytic domain, has a leucine/isoleucine-rich C-terminus and a tandem structural homology of the ZBD in its C-terminus. APOBEC-2 deaminated free nucleotides *in vitro* but did not have editing activity on
25 apoB mRNA.

 Human phorbolin 1, phorbolin 1-related protein, phorbolin-2 and -3 share characteristics with C to U editing enzymes. Several proteins with homology to APOBEC-1 named Phorbolins 1, 2, 3, and Phorbolin-1 related protein were identified in skin from patients suffering from psoriasis and were shown to be induced (in the case of Phorbolins 1 and 2) in skin treated with
30 phorbol 12-myristate-1-acetate (Muramatsu, M. et al. (1999) J Biol Chem. 274:18470-6). The genes for these proteins were subsequently renamed as members of the APOBEC-3 or ARP family locus (Table 1) (Madsen, P. et al. (1999) J Invest Dermatol. 113:162-9). Bioinformatic studies revealed the presence of two additional APOBEC-1 related proteins in the human genome. One is an expressed gene (XM_092919) located just 2 kb away from APOBEC-3G,

and is thus likely to be an eighth member of the family. The other is at position 12q23, and has similarity to APOBEC-3G.

ARP variants show homology to cytidine deaminases (Figure 2d). As anticipated from the SBSA, Some of these proteins bind zinc and have RNA binding capacities similar to APOBEC-1 Jarmuz, A., *et al.*, (2002) Genomics, 79:285-96). However, analysis of APOBEC-3A, -3B and -3G revealed them unable to edit apoB mRNA Jarmuz, A., *et al.*, (2002) Genomics, 79:285-96); Muramatsu, M. *et al.* (1999) J Biol Chem. 274:18470-6). It has been shown that the frequency of deleterious mutations in HIV and impaired infectivity correlated with the expression of CEM15 (APOBEC-3G) (Sheehy *et al*, 2002; Mariani *et al*, 2003; Mangeat *et al*, 2003; Harris *et al*, 2003; Lecossier *et al*, 2003.

Table 1.

Gene/Chromosomal location	Protein Accession #	Equivalent/Former Names/Variants (Accn #)	Expression	Proposed CDAR/ARP
Yeast				
CDD1/Chr XII	NP_013346	-	yeast	ScCDAR-1
Human				
APOBEC-1/12p13.1	AAD00185	-	small intestine, liver	HsCDAR-1
APOBEC-2/6p21	NP_006780	CAB44740 ARCD-1	cardiac & skeletal muscle	HsARP-1
AID/12p13	NP_065712	-	B lymphocytes	HsARP-2
APOBEC-3A/22q13.1	NP_663745	Phorbolin-1 (P31941)	keratinocytes	HsARP-3
APOBEC-3B/22q13.1	Q9U1117	Phorbolin-3, Phorbolin-1-related (U61084), Phorbolin-2 (Q9UE74), APOBECIL, ARCD-3	keratinocytes/ colon	HsARP-4
APOBEC-3C/22q13.1	CAB-15271	Phorbolin-1 (AF165520), ARCD-2/ARCD-4	spleen/testes/heart/ thymus/prostate/ovary/ uterus/PBLs	HsARP5
APOBEC-3D/22q13.1	BF841711 (EST only)	-	hHead & neck cancers	HsARP-6
APOBEC-3E/22q13.1	pseudogene	ARCD-6	-	-
APOBEC-3D*3E/22q13.1	NM_145298	-	uterus	HsARP-7
APOBEC-3F/22q13.1	BG_758984 (EST only)	ARCD-5	B lymphocytes	HsARP-8
APOBEC-3G/22q13.1	NP_068594	Phorbolin-like-protein, MDS019 (AAH24268), HsCEM15	spleen/testes/heart/ thymus/PBLs/colon/ stomach/kidney/uterus/ pancreas/placenta/ prostate	HsARP-9
22q13.1	XP_092919	-	-	HsARP-10
12q23	XP_115170	-	-	HsARP-11
Mouse				
Mm APOBEC-1/6F2	NP_112436	-	small intestine/liver/ spleen/B lymphocytes/ kidney	MmCDAR-1
Mm APOBEC-2/17	NP_033824	-	cardiac & skeletal muscle/hair/skin	MmARP-1
mmAID/6F2	NP_033775	-	B lymphocytes	MmARP-2
CEM15/15	NP_084531	XP_122858	mammary tumor	MmARP-3

HIV expressing functional Vif (viral infectivity factor) protein is able to overcome the effects of CEM15 due to the ability of Vif to bind and target for ubiquitination and destruction in the proteasome (Mariani et al., Cell 114:21-31, 2003; Stopal et al. Mol. Cell 12:591-601, 2003; Yu et al. Nat Struct Mol. Biol 11:435-42, 2004). In contrast, it is unlikely that APOBEC-3D and 3E function as an APOBEC-1 like editases because they are missing fundamental sequence elements that are required for mRNA editing by both APOBEC-1 and CDD1 (Anant, S. et al. (2001) Am J Physiol Cell Physiol. 281:C1904-16; Dance et al 2001), and experimental evidence shows an impaired ability to coordinate Zn^{2+} and deaminate cytidine Jarmuz, A., et al., (2002) Genomics, 79:285-96). APOBEC-3E appears to be a pseudogene (Jarmuz, A., et al., (2002) Genomics, 79:285-96), yet the EST database shows that APOBEC-3D and APOBEC-3E are alternatively spliced to form a single CD-PCD-CD-PCD encoding transcript.

Additionally, it has been shown that rat APOBEC-1, mouse APOBEC-3, and human APOBEC-3B, are able to inhibit HIV infectivity even in the presence of Vif. Like APOBEC-3G, human APOBEC-3F preferentially restrict vif-deficient virus. The mutation spectra and expression profile of APOBEC-3F indicate that this enzyme, together with APOBEC-3G, accounts for the G to A hypermutation of proviruses described in HIV-infected individuals (Bishop et al., Curr. Bio. 14:1392-1396, 2004). In accordance with this, it has also been shown that APOBEC-3F blocks HIV-1 and is suppressed by both the HIV-1 and HIV-2 Vif proteins (Zheng et al, J Virol 78(11): 6073-6076, 2004; Wiegand et al, EMBO 23:2451-58, 2004). The limited tissue expression, and association with pre-cancerous and cancerous cells (Table 1), and in the case of APOBEC-3G, antagonism of the HIV viral protein Vif shows specific roles for the APOBEC-3 family in growth/cell cycle regulation and antiviral control.

CEM15 (APOBEC-3G) has also been shown to interfere with other retroelements, including but not limited to hepatitis B virus (HBV) and murine leukemia virus (MLV). The methods and compositions described herein are useful with any of these viruses (Bishop et al., Curr. Bio. 14:1392-1396, 2004; Machida et al., PNAS 101(12):4262-67, 2004; Turelli et al., Science, 303:1829, 2004).

Human HIV-1 virus contains a 10-kb single-stranded, positive-sense RNA genome that encodes three major classes of gene products that include: (i) structural proteins such as Gag, Pol and Env; (ii) essential *trans*-acting proteins (Tat, Rev); and (iii) "auxiliary" proteins that are not required for efficient virus replication in at least some cell culture systems (Vpr, Vif, Vpu, Nef). Among these proteins, Vif is required for efficient virus replication *in vivo*, as well as in certain host cell types *in vitro* (Fisher et al. Science 237(4817):888-93, 1987; Strebel et al. Nature

328(6132):728-30, 1987) because of its ability to overcome the action of a cellular antiviral system (Madani et al. J Virol 72(12):10251-5, 1998; Simon et al. Nat Med 4(12):1397-400, 1998).

The *in vitro* replicative phenotype of *vif*-deleted molecular clones of HIV-1 is strikingly different in *vif*-permissive cells (e.g. 293T, SUPT1 and CEM-SS T cell lines), as compared to *vif*-non-permissive cells (e.g. primary T cells, macrophages, or CEM, H9 and HUT78 T cell lines). In the former cells, *vif*-deleted HIV-1 clones replicate with an efficiency that is essentially identical to that of wild-type virus, whereas in the latter cells, replication of *vif*-negative HIV-1 mutants is arrested due to a failure to accumulate reverse transcripts and inability to generate infectious proviral integrants in the host cell (Sova et al. J Virol 67(10):6322-6, 1993; von Schwedler et al. J Virol 67(8):4945-55, 1993; Simon et al. J Virol 70(8):5297-305, 1996; Courcoul et al. J Virol 69(4):2068-74, 1995). These defects are due to the expression of the host protein CEM15 (Sheehy, A.M., et al., (2002) Nature. 418:646-650) in non-permissive cells for *vif* minus viruses. CEM15 antiviral activity is derived from effects on viral RNA or reverse transcripts (Sheehy, A.M., et al., (2002) Nature. 418:646-650). CEM15 deaminates dC to dU as the first strand of DNA is being made by reverse transcriptase or soon after its completion, and this results in dG to dA changes at the corresponding positions during second strand DNA synthesis (Harris et al. Cell 113:803-809, 2003).

Primary sequence alignments (Figure 3) and the structural constraints relating CDAs to APOBEC-1 indicate that CEM15 evolved from an APOBEC-1-like precursor by gene duplication (Wedekind et al. Trends Genet 19(4): p. 207-16, 2003). The resulting CEM15 structure exhibits two active sites per polypeptide chain with the topology CD1-PCD1-connector-CD2-PCD2. Knowledge of the structural homology among CDAs and ARPs is sufficient to understand how features of CEM15 contribute to its anti-viral activity.

Vif interacts with CEM15 and induces its poly-ubiquitination and degradation through the proteasome, thereby reducing the abundance of CEM15 and promoting viral infectivity. It has been discovered that Vif homodimers were required for Vif's interaction with CEM15 (Yang et al. J Biol Chem. 278(8): 6596-602 (2003) and US Patent 6,653,443, herein incorporated by reference in their entirety).

Stably expressed CEM15 significantly reduced the level of pseudotyped HIV-1 particles lacking Vif. The reduced viral particle production is the result of a selective suppression of viral RNA leading to reduction in essential HIV-1 proteins. These effects were not observed when Vif was expressed due to the marked reduction of CEM15. Although CEM15 was required to deplete viral particle production its deaminase function was not necessary. The

data indicate an antiviral mechanism in producer cells which is potentially significant late during the viral life cycle that involves directly or indirectly the RNA binding ability of CEM15 and does not require virion incorporation of CEM15 deaminase activity during viral replication.

Thus, agents that enhance CEM15 selective binding to viral RNA, leading to viral RNA

destruction result in a reduction in viral particle production and a reduced viral burden for the subject.

Disclosed herein are methods of predicting the severity of a viral infection in a subject. In one embodiment, the method of predicting the severity of a viral infection in a subject comprises the steps of acquiring a biological sample from the subject; and measuring the level of expression of one or more APOBEC-1 related proteins in the subject, wherein a higher level of expression as compared to a reference level indicates decreased severity. The reference level is a selected control level. Alternatively, the reference level can be from a severely infected subject(s) and a lower level of expression in the test subject would indicate a less severe viral infection.

As disclosed above, greater amounts of APOBEC-1 related proteins can indicate a less severe viral infection. CEM15 contributes to the control of viral replication (Sheehy et al. (2002) Nature 418:646-650; Mariani et al. (2002) Cell 114:21-31). A number of studies have examined the role of CEM15 in suppressing HIV replication *in vitro* (Harris et al. (2003) Cell 113:803-809; Zhang et al. (2003) Nature 424:94-98; Mangeat et al. (2003) Nature 424:99-103). Also, CEM15 genetic variants can influence HIV disease progression (An et al. (2004) J. Virol. 78:11070-11076). Increased CEM15 gene expression provides a competitive advantage in that viral Vif is not able to destroy all of the enzyme prior to each round of infection and packaging, and consequently over time mutations in the HIV genome accumulate to the point of debilitating the virus and suppressing infectivity. Consequently, a slower rate of HIV disease progression is observed in patients with elevated CEM15 expression capacity.

In the method described above, the test subject can have a viral infection when the levels of expression are measured. Alternatively, the subject may be free of the viral infection in question, and still be tested to determine the likely response of the subject to a potential viral infection.

Decreased severity can result in an increased longevity in the subject as compared to a control. For example, if greater levels of an APOBEC-1 related protein are found, the individual can be expected to live 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 months longer, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more years longer compared to a control. The decreased severity can also comprise a longer asymptomatic period in the subject as

compared to a control. For example, the subject can remain asymptomatic for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 months longer, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more years longer compared to a control. Further, the decreased severity can result in reduced symptoms of the viral infection (e.g., reduced fever, reduced inflammation, and reduced secondary infections.)

The decreased severity can be manifest in a number of different ways. For example, the decreased severity can comprise high CD4 counts as compared to a control. The CD4 count has been used as a measurement to determine the strength of the immune system. It can also be used to judge how far a viral infection is advanced (the stage of the disease), and helps predict the risk of complications and opportunistic infections. The CD4 count can be compared with a count obtained from an earlier test in the same subject. The CD4 count can also be used in combination with the viral load test, which measures the level of HIV in the blood, to determine the staging and outlook of the disease. A CD4 count and a viral load test are usually ordered when a subject is diagnosed with a virus, such as HIV, as part of a baseline measurement. Both tests are commonly repeated about four weeks after starting anti-HIV therapy. If treatment is maintained, a CD4 count can be performed every three to four months thereafter, for example.

Normal CD4 counts in adults range from 500 to 1,500 cells per cubic millimeter of blood. In general, the CD4 count goes down as the viral disease progresses. According to public health guidelines, preventive therapy should be started when an HIV-positive person who has no symptoms registers a CD4 count under 350. The Centers for Disease Control and Prevention considers HIV-infected persons who have CD4 counts below 200 to have AIDS, regardless of whether they are symptomatic.

The decreased severity can also comprise lower HIV viremia levels as compared to a control. Quantitative measurements of HIV viremia in peripheral blood have shown that higher virus levels can be correlated with increased risk of clinical progression of HIV disease, and that reductions in plasma virus levels can be associated with decreased risk of clinical progression. Virus levels in the peripheral blood can be quantitated by direct measurement of viral RNA in plasma using nucleic acid amplification technologies, such as the polymerase chain reaction assay, branched DNA assay and nucleic acid sequence-based amplification assay. These assays quantify human immunodeficiency virus (HIV) RNA levels. Plasma viral load (PVL) testing has become a cornerstone of HIV disease management. Initiation of antiretroviral drug therapy is usually recommended when the PVL is 10,000 to 30,000 copies per mL or when CD4+ T-lymphocyte counts are less than 350 to 500 per mm³ (0.35 to 0.50 $\times 10^9$ per L). PVL levels usually show a 1- to 2-log reduction within four to six weeks after therapy is started. The goal is

no detectable virus in 16 to 24 weeks. Periodic monitoring of PVL is important to promptly identify treatment failure. The same assay can be used for serial PVL testing in the subject. At least two PVL measurements are usually performed before antiretroviral drug therapy is initiated or changed.

5 Stably expressed CEM15 significantly reduced the level of pseudotyped HIV-1 particles lacking Vif. The reduced viral particle production is the result of a selective suppression of viral RNA leading to reduction in essential HIV-1 proteins. These effects were not observed when Vif was expressed due to the marked reduction of CEM15. The data indicate an antiviral mechanism in producer cells which is potentially significant late during the viral life
10 cycle that involves directly or indirectly the RNA binding ability of CEM15 and does not require virion incorporation of CEM15 deaminase activity during viral replication.

One of ordinary skill in the art at the time of the invention would know how to measure either DNA, mRNA or protein. For example, they can be measured using a blood sample, a cellular extract, or a tissue extract. Urine samples can also be used.

15 Also disclosed are methods of predicting whether a subject is or will be a long term nonprogressor (LTNP) when infected with a virus. In one embodiment, this method comprises acquiring a biological sample from the subject; and measuring the level of expression of one or more APOBEC-1 related proteins in the subject, wherein a higher level of expression as compared to a reference level (e.g., normal level) indicates the subject is a potential LNTP. If the
20 reference level is that of a rapid progressor, then the difference in the levels may be greater.

A small subset of HIV-infected individuals, known as long-term nonprogressors (LNTNs) have substantially slower rates of disease progression in the absence of therapeutic intervention. Clinically, these LNTNs are usually asymptomatic, maintain high CD4 counts and low HIV viremia levels. The characteristics are therefore of prognostic value in evaluating
25 disease severity. The mechanisms responsible for long-term nonprogression have previously been attributed to defective or less fit HIV variants, strong host immune responses and unique host genetic elements, such as the CCR5 genotype and HLA haplotypes (Buchbinder et al. (1999) *Microbes and Infection* 1:1113-1120). As disclosed in Example 1, these LNTNs are associated with higher levels of APOBEC-1 related proteins.

30 As disclosed above, the indication of a LTNP can be manifested in a number of different ways. For example, the decreased severity can comprise high CD4 counts as compared to a control. The decreased severity can also comprise lower HIV viremia levels as compared to a control.

In the methods described above, the subject can have a viral infection when the levels of expression are measured. Alternatively, the subject may be free of the viral infection in question, and still be tested to determine the likely response of the subject as a potential LTNP. The viral infection can be a lentiviral infection, such as HIV-1.

5 The RNA virus can be selected from the list of viruses consisting of Vesicular stomatitis virus, Hepatitis A virus, Hepatitis C virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, 10 St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Hantavirus, and Rubella virus.

 Also disclosed herein are methods of optimizing antiviral therapy in a subject with a viral infection. In one embodiment, the method comprises the steps of acquiring a biological sample from the subject; detecting the level of expression of one or more APOBEC-1 related 15 proteins in the sample; and adjusting the antiviral therapy according to the levels APOBEC-1 related proteins, thereby optimizing the viral therapy. If the antiviral therapy is associated with high levels of ARP, this is desired.

 There are many types of antiviral therapy available. These therapies include, but are not limited to, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase 20 inhibitors, nucleotide reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, integrase inhibitors, or any combination thereof.

 The antiviral therapy can be reduced when the expression levels of APOBEC-1 related proteins is high as compared to a reference level. Many of the therapies available to those with a viral infection are expensive and have undesirable side effects. If a subject is expressing high 25 levels of APOBEC-1 related proteins, antiviral therapy can be reduced accordingly, thereby making treatment options customizable to the subject in need thereof. Alternatively, the antiviral therapy can be increased when the expression levels of APOBEC-1 related proteins is low as compared to a reference level. If the levels are found to be below a normal range, or an optimal amount, the treatment can be increased accordingly.

30 Also disclosed are methods of predicting the level of CD4 cells in a subject, comprising acquiring a biological sample from the subject; and detecting the level of CEM15 expression in the subject, the level of CEM15 correlating with the level of CD4 cells. As disclosed above, the CD4 count has been used as a measurement to determine the strength of the immune system. It

can also be used to judge how far a viral infection is advanced (the stage of the disease), and helps predict the risk of complications and opportunistic infections.

Also disclosed is a method of monitoring effectiveness of an antiviral agent in a subject. In one embodiment, these steps comprise detecting expression levels of one or more APOBEC-1 related proteins in a first biological sample from the subject prior to administration of the agent; and detecting expression levels of one or more APOBEC-1 related proteins in a second or any subsequent biological sample from the subject after administration of the agent, an increase in expression levels of the APOBEC-1 related proteins in the second or subsequent sample as compared to the first sample indicating effectiveness of the antiviral agent.

In one example, the agent administered to the subject targets a Vif/CEM15 interaction. The agent can be, for example, a Vif antagonist or a cytidine deaminase activator. The agent can also be selected from the group consisting of nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors, protease inhibitor, and fusion inhibitors, or a combination thereof.

Also disclosed herein are methods for correlating a specific anti-viral therapy with CEM15 levels in a subject. For example, disclosed is a method of treating a subject infected with a virus with an appropriate antiviral agent, comprising the steps of: identifying a population of subjects with a given range of APOBEC-1 related protein levels; determining which antiviral agent is most effective at the given range of APOBEC-1 related protein levels; and administering an appropriate antiviral agent to the subject in need thereof. Therefore, treatment options can be customized to an individual based on their specific ARP level. By so doing, subjects can be treated based on their specific needs. High levels of CEM15, for example, can dictate that the subject is in need of one type of therapy, while low levels of CEM15 can indicate that a different type of therapy would be more effective. One of ordinary skill in the art is able to determine ARP ranges. These levels can then be coordinated to a given treatment therapy, as disclosed herein.

In the methods disclosed herein, the APOBEC-1 related proteins can be selected from the group consisting of CEM15, APOBEC-3B, APOBEC-3C, APOBEC-3F. APOBEC-3F, has potent activity against virion infectivity factor deficient (Δ vif) human immunodeficiency virus 1 (HIV-1). These enzymes become encapsidated in Δ vif HIV-1 virions and in the next round of infection deaminate the newly synthesized reverse transcripts. APOBEC-3B and APOBEC-3C have potent antiviral activity against simian immuno-deficiency virus (SIV). Both enzymes were encapsidated in SIV virions and were active against Δ vif SIV(mac) and SIV(agm). APOBEC-3B induced abundant G to A mutations in both wild-type and Δ vif SIV reverse transcripts.

APOBEC-3C induced substantially fewer mutations. APOBEC-3F was found to be active against SIV and sensitive to SIV(mac) Vif. (Yu et al. J Biol Chem. (2004) Dec 17;279(51):53379-86.)

Expression of the APOBEC-1 related protein can be measured by detecting DNA, mRNA or protein levels of the APOBEC-1 related protein. For example, mRNA levels are detected by PCR, such as real time PCR (rtPCR).

PCR is useful for obtaining quantitative information about the expression of many different genes in a sample that can contain as little as a single cell. Since the disclosed methods are quantitative, comparisons of the expression patterns at a quantitative level between a variety of different cell states or cell types can be achieved. In general, total RNA can be isolated from the target sample using any isolation procedure. This RNA can then be used to generate first strand copy DNA (cDNA) using any procedure, for example using random primers or oligo-dt primers or random-oligo-dt primers which are oligo-dT primers coupled, on the 3' end, to short stretches of specific sequence covering all possible combinations, so the primer primes at the junction between the polyA tract and non-poly A tract associated with messenger RNA (mRNA). The cDNA is then used as a template in a PCR reaction. This PCR reaction is performed with primer sets, a forward and a reverse primer, that are specific for the expressed genes, which are to be tracked.

A real time PCR protocol can be used with the methods disclosed herein. These methods, for example, rely on increases in fluorescence at each cycle of PCR through, for example, the release of fluorescein from a quencher sequence while the uniprimer (universal primer) binds to the DNA sequence. Fluorescence approaches used in real-time quantitative PCR are typically based on a fluorescent reporter dye such as SYBR green, FAM, fluorescein, HEX, TET, TAMRA, etc. and a quencher such as DABSYL or Black Hole, for example. When the quencher is separated from the probe during the extension phase of PCR, the fluorescence of the reporter can be measured. Systems like Molecular Beacons, Taqman Probes, Scorpion Primers or Sunrise Primers and others use this approach to perform real-time quantitative PCR. Examples of methods and reagents related to real time probes can be found in United States Patent Nos: 5,925,517; 6,103,476; 6,150,097, and 6,037,130, which are incorporated by reference herein at least for material related to detection methods for nucleic acids and PCR methods.

The cDNA sequences of APOBEC-3B (SEQ ID NO: 9), APOBEC-3C (SEQ ID NO: 11), APOBEC-3F (SEQ ID NO: 13) and CEM15 (SEQ ID NO: 5) are highly homologous but have several stretches of non-identity that can be used in the design of specific primers and/or probes for the selective real time PCR quantification of each homolog. APOBEC-3C is half the

size of APOBEC-3B, APOBEC-3F and CEM15 and is homologous to only the 3' portion of these transcripts. Consequently, primer and probe combinations within the 5' half of APOBEC-3B, APOBEC-3F and CEM15 does not amplify APOBEC-3C. Importantly APOBEC-3C cDNA sequence between nucleotides 1-194 are not well conserved with comparable regions within the 3' half of APOBEC-3B, APOBEC-3F and CEM15 and therefore can be used in the design of primer and probes for the selective amplification and quantification of APOBEC-3C. For example, SEQ ID NO: 17 discloses nucleotides 1-194 of APOBEC-3C and this sequence, along with fragments or portions thereof, can be used to specifically amplify or detect APOBEC-3C.

Regions of APOBEC-3F sequence with significant divergence that has utility in the selective amplification and quantification this cDNA are apparent from nucleotides 1-60 and 1328-1725 (SEQ ID NOS: 18 and 19). Moreover, APOBEC-3F has a unique 1000 nucleotide long 3' untranslated region that has utility in quantifying this cDNA. SEQ ID NOS: 18 and 19, or fragments or portions thereof, can be used to specifically amplify or detect APOBEC-3F.

APOBEC-3B sequence divergence that has utility in the selective amplification and quantification this cDNA are apparent from nucleotides 1-67 and 910-1007 (SEQ ID NOS 15 and 16). SEQ ID NOS: 15 and 16, or fragments or portions thereof, can be used to specifically amplify or detect APOBEC-3B.

Expression can also be measured by detecting protein levels of APOBEC-1 related proteins. Such detection can occur, for example, by Western blotting. CEM15 protein levels can also be detected using ELISA. Those of skill in the art know how to quantify protein levels using Western blotting or ELISA techniques.

Disclosed herein are methods of screening for an antiviral agent, comprising administering to a subject with a viral infection an agent to be screened; and detecting expression levels of one or more APOBEC-1 related proteins in a biological sample from the subject, an increased expression level indicating an antiviral agent.

As discussed above, an "increased expression level" means an increase in the level of the APOBEC-1 related protein as compared to a control. Therefore, the antiviral agent inhibits or suppresses viral infectivity. An "inhibitor" or "suppressor" can be anything that reduces activity. If the amount of CEM15 is increased in the presence of the composition as compared to the amount of CEM15 in the absence of the composition, the composition can be said to increase the expression level of CEM15.

The screening methods disclosed herein can be used with a high throughput screening assay, for example. The high throughput assay system can comprise an immobilized array of test compounds. Alternatively, the Vif molecule or the cytidine deaminase molecule can be

immobilized. There are multiple high throughput screening assay techniques that are well known in the art (for example, but not limited to, those described in Abriola et al., J. Biomol. Screen 4:121-127, 1999; Blevitt et al., J. Biomol. Screen 4:87-91, 2000; Hariharan et al., J. Biomol. Screen 4:187-192, 1999; Fox et al., J. Biomol. Screen 4:183-186, 1999; Burbaum and Sigal, Curr. Opin. Chem. Biol. 1:72-78, 1997; Jayasena, Clin. Chem. 45:1628-1650, 1999; and Famulok and Mayer, Curr. Top. Microbiol. Immunol. 243:123-136, 1999).

Agents with antiviral activity can be identified from large libraries of natural products or synthetic (or semi-synthetic) extracts or chemical libraries according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds (e.g., but not limited to, antibodies, peptides, and aptamers). Synthetic compound libraries are commercially available, e.g., from Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI).

The ability of a test compound to enhance CEM15 expression can be measured by contacting the test compound with a cell in the presence of CEM15, either *in vivo* or *in vitro*. The CEM15 function can be, but is not limited to, its cytidine to uridine editing of RNA, or its deoxycytidine to deoxyuridine mutation of DNA, or its suppression of viral activity, or its activity on cancerous or precancerous cells. An "increase in CEM15" is defined as a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 2-fold, 10-fold, 100-fold, or 1000-fold increase in the amount of the CEM15. Also contemplated is an increase in the activity of CEM15.

Disclosed herein are primers, probes, and nucleic acid sequences corresponding to proteins thereof, such as Vif and the ARP family of proteins. For example SEQ ID NOS: 15-16 can be used to amplify APOBEC-3B.

It is understood that as discussed herein the use of the terms homology and identity mean the same thing as similarity. Thus, for example, if the use of the word homology is used between two non-natural sequences it is understood that this is not necessarily indicating an evolutionary relationship between these two sequences, but rather is looking at the similarity or relatedness between their nucleic acid sequences. Many of the methods for determining

homology between two evolutionarily related molecules are routinely applied to any two or more nucleic acids or proteins for the purpose of measuring sequence similarity regardless of whether they are evolutionarily related or not.

5 In general, it is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed genes and proteins herein, is through defining the variants and derivatives in terms of homology to specific known sequences. This identity of particular sequences disclosed herein is also discussed elsewhere herein. In general, variants of genes and proteins herein disclosed typically have at least, about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent homology to
10 the stated sequence or the native sequence. Those of skill in the art readily understand how to determine the homology of two proteins or nucleic acids, such as genes. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

Another way of calculating homology can be performed by published algorithms.
15 Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. Mol Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin
20 Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein
25 incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods may differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity, and be disclosed herein.

30 For example, as used herein, a sequence recited as having a particular percent homology to another sequence refers to sequences that have the recited homology as calculated by any one or more of the calculation methods described above. For example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using the Zuker calculation method even if

the first sequence does not have 80 percent homology to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using both the Zuker calculation method and the
5 Pearson and Lipman calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent
10 homology to the second sequence using each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

The term hybridization typically means a sequence driven interaction between at least two nucleic acid molecules, such as a primer or a probe and a gene. Sequence driven interaction means an interaction that occurs between two nucleotides or nucleotide analogs or nucleotide
15 derivatives in a nucleotide specific manner. For example, G interacting with C or A interacting with T are sequence driven interactions. Typically sequence driven interactions occur on the Watson-Crick face or Hoogsteen face of the nucleotide. The hybridization of two nucleic acids is affected by a number of conditions and parameters known to those of skill in the art. For example, the salt concentrations, pH, and temperature of the reaction all affect whether two
20 nucleic acid molecules will hybridize.

Parameters for selective hybridization between two nucleic acid molecules are well known to those of skill in the art. For example, in some embodiments selective hybridization conditions can be defined as stringent hybridization conditions. For example, stringency of hybridization is controlled by both temperature and salt concentration of either or both of the
25 hybridization and washing steps. For example, the conditions of hybridization to achieve selective hybridization may involve hybridization in high ionic strength solution (6X SSC or 6X SSPE) at a temperature that is about 12-25°C below the T_m (the melting temperature at which half of the molecules dissociate from their hybridization partners) followed by washing at a combination of temperature and salt concentration chosen so that the washing temperature is
30 about 5°C to 20°C below the T_m . The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to a labeled nucleic acid of interest and then washed under conditions of different stringencies. Hybridization temperatures are typically higher for DNA-RNA and RNA-RNA hybridizations. The conditions can be used as described above to achieve stringency, or as

is known in the art. (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989; Kunkel et al. Methods Enzymol. 1987:154:367, 1987 which is herein incorporated by reference for material at least related to hybridization of nucleic acids). A preferable stringent hybridization condition for a DNA:DNA hybridization can be at about 68°C (in aqueous solution) in 6X SSC or 6X SSPE followed by washing at 68°C. Stringency of hybridization and washing, if desired, can be reduced accordingly as the degree of complementarity desired is decreased, and further, depending upon the G-C or A-T richness of any area wherein variability is searched for. Likewise, stringency of hybridization and washing, if desired, can be increased accordingly as homology desired is increased, and further, depending upon the G-C or A-T richness of any area wherein high homology is desired, all as known in the art.

Another way to define selective hybridization is by looking at the amount (percentage) of one of the nucleic acids bound to the other nucleic acid. For example, in some embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the limiting nucleic acid is bound to the non-limiting nucleic acid. Typically, the non-limiting primer is in for example, 10 or 100 or 1000 fold excess. This type of assay can be performed at under conditions where both the limiting and non-limiting primer are for example, 10 fold or 100 fold or 1000 fold below their k_d , or where only one of the nucleic acid molecules is 10 fold or 100 fold or 1000 fold or where one or both nucleic acid molecules are above their k_d .

Another way to define selective hybridization is by looking at the percentage of primer that gets enzymatically manipulated under conditions where hybridization is required to promote the desired enzymatic manipulation. For example, in some embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer is enzymatically manipulated under conditions which promote the enzymatic manipulation, for example if the enzymatic manipulation is DNA extension, then selective hybridization conditions would be when at least about 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer molecules are extended. Preferred conditions also include those suggested by the manufacturer or indicated in the art as being appropriate for the enzyme performing the manipulation.

Just as with homology, it is understood that there are a variety of methods herein disclosed for determining the level of hybridization between two nucleic acid molecules. It is

understood that these methods and conditions may provide different percentages of hybridization between two nucleic acid molecules, but unless otherwise indicated meeting the parameters of any of the methods would be sufficient. For example if 80% hybridization was required and as long as hybridization occurs within the required parameters in any one of these methods it is considered disclosed herein.

It is understood that those of skill in the art understand that if a composition or method meets any one of these criteria for determining hybridization either collectively or singly it is a composition or method that is disclosed herein.

There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode primers and probes. The disclosed nucleic acids are made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell, that the expressed mRNA will typically be made up of A, C, G, and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through for example exogenous delivery, it is advantageous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. An non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

A nucleotide analog is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to nucleotides are well known in the art and would include for example, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, and 2-aminoadenine as well as modifications at the sugar or phosphate moieties.

Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety.

Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety. (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989,86, 6553-6556),

A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH₂ or O) at the C6 position of purine nucleotides.

Sequences

There are a variety of sequences related to, for example, CEM15 as well as any other protein disclosed herein that are disclosed on Genbank, and these sequences and others are herein incorporated by reference in their entireties as well as for individual subsequences contained therein.

A variety of sequences are provided herein and these and others can be found in Genbank, at www.pubmed.gov. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences. Primers and/or probes can be designed for any sequence given the information disclosed herein and known in the art.

Disclosed are compositions including primers and probes, which are capable of interacting with the genes disclosed herein. In certain embodiments the primers are used to support DNA amplification reactions. Typically the primers will be capable of being extended in a sequence specific manner. Extension of a primer in a sequence specific manner includes any methods wherein the sequence and/or composition of the nucleic acid molecule to which the primer is hybridized or otherwise associated directs or influences the composition or sequence of the product produced by the extension of the primer. Extension of the primer in a sequence specific manner therefore includes, but is not limited to, PCR, DNA sequencing, DNA

extension, DNA polymerization, RNA transcription, or reverse transcription. Techniques and conditions that amplify the primer in a sequence specific manner are preferred. In certain embodiments the primers are used for the DNA amplification reactions, such as PCR or direct sequencing. It is understood that in certain embodiments the primers can also be extended using
5 non-enzymatic techniques, where for example, the nucleotides or oligonucleotides used to extend the primer are modified such that they will chemically react to extend the primer in a sequence specific manner. Typically the disclosed primers hybridize with the nucleic acid or region of the nucleic acid or they hybridize with the complement of the nucleic acid or complement of a region of the nucleic acid.

10 Disclosed is an isolated nucleic acid sequence comprising a sequence at least 80% identical to SEQ ID NO: 1 (5'CGCAGCCTGTGTCAGAAAAG3'). The nucleic acid sequence comprising the nucleotide sequence of SEQ ID NO: 1, or variants or fragments thereof, wherein the variant or fragment comprises a specific CEM15 primer. Also contemplated is an isolated nucleic acid sequence comprising at least five consecutive nucleotides of SEQ ID NO: 1,
15 wherein the nucleic acid sequence comprises a specific CEM15 primer. Also contemplated is an isolated nucleic acid sequence comprising the sequence of SEQ ID NO: 1.

Disclosed herein is an isolated nucleic acid sequence comprising a sequence at least 80% identical to SEQ ID NO: 2 (5'CCAACAGTGCTGAAATTCGTCATA3'). Contemplated herein is an isolated nucleic acid sequence comprising the nucleotide sequence of SEQ ID NO: 2, or
20 variants or fragments thereof, wherein the variant or fragment comprises a specific CEM15 primer. Described herein is an isolated nucleic acid sequence comprising at least five consecutive nucleotides of SEQ ID NO: 2, wherein the nucleic acid sequence comprises a specific CEM15 primer. Further disclosed is an isolated nucleic acid sequence comprising SEQ ID NO: 2.

25 Disclosed herein is an isolated nucleic acid sequence comprising a sequence at least 80% identical to SEQ ID NO: 3 (5'GTGCCACCATGAAGA3'). Described herein is an isolated nucleic acid sequence comprising the nucleotide sequence of SEQ ID NO: 3, or variants or fragments thereof, wherein the variant or fragment comprises a specific CEM15 probe. Also described is an isolated nucleic acid sequence comprising at least five consecutive nucleotides of
30 SEQ ID NO: 3, wherein the nucleic acid sequence comprises a specific CEM15 probe.

Disclosed are antiviral agents identified by the screening methods disclosed herein. The antiviral agent can increase the expression level of CEM15 in a subject. Alternatively, the antiviral agent can bind, or otherwise interact, with a cytidine deaminase or deoxycytidine deaminase, thereby enhancing the normal activity of the cytidine deaminase or deoxycytidine

deaminase. For example, a cytidine deaminase activator can interact with CEM15 and enhance the binding of CEM15 to a virus. Conversely, a cytidine deaminase activator can interact with the binding of Vif to a CEM15 molecule, thereby suppressing the activity of Vif, and indirectly enhancing CEM15 binding to HIV.

5 In the methods disclosed herein, molecules such as CEM15 and Vif can be used in assays. These molecules can be, for example, chimeric proteins. By “chimeric protein” is meant any single polypeptide unit that comprises two distinct polypeptide domains joined by a peptide bond, optionally by means of an amino acid linker, or a non-peptide bond, wherein the two domains are not naturally occurring within the same polypeptide unit. Typically, such chimeric
10 proteins are made by expression of a cDNA construct but could be made by protein synthesis methods known in the art. These chimeric proteins are useful in screening compounds, as well as with the compounds identified by the methods disclosed herein.

The compositions disclosed herein can also be fragments or derivatives of a naturally occurring deaminase or viral infectivity factor. A “fragment” is a polypeptide that is less than the
15 full length of a particular protein or functional domain. By “derivative” or “variant” is meant a polypeptide having a particular sequence that differs at one or more positions from a reference sequence. The fragments or derivatives of a full length protein preferably retain at least one function of the full length protein. For example, a fragment or derivative of a deaminase includes a fragment of a deaminase or a derivative deaminase that retains at least one binding or
20 deaminating function of the full length protein. By way of example, the fragment or derivative can include a Zinc-Dependent Cytidine Deaminase domain or can include 20, 30, 40, 50, 60, 70 80, 90% similarity with the full length deaminase. The fragment or derivative can include conservative or non-conservative amino acid substitutions. The fragment or derivative can include a linker sequence joining a catalytic domain (CD) to a pseudo-catalytic domain (PCD)
25 and can have the domain structure CD-PCD-CD-PCD or any repeats thereof. The fragment or derivative can comprise a CD. Other fragments or derivatives are identified by structure-based sequence alignment (SBSA) as shown herein. See Figure 2b that reveals the consensus structural domain attributes of APOBEC-1 and ARPs (Figure 2c). The fragment or derivative optionally can form a homodimer or a homotetramer. Also disclosed are chimeric proteins,
30 wherein the deaminase domain is a fragment or derivative of CEM15 having deaminase function.

“Deaminases” include deoxycytidine deaminase, cytidine deaminase, adenosine deaminase, RNA deaminase, DNA deaminase, and other deaminases. Optionally, the deaminase is APOBEC-1 (see international patent application designated PCT/US02/05824, which is

incorporated herein by reference in its entirety for APOBEC-1, chimeric proteins related thereto, and uses thereof) (Gen Bank Accession # NP_001635), REE (see U.S. Pat. No. 5,747,319, which is incorporated herein by reference in its entirety for REE and uses thereof), or REE-2 (see U.S. Pat. No. 5,804,185, which is incorporated herein by reference in its entirety for REE-2 and uses thereof). Deaminases as described herein can include the following structural features: three or more CDD-1 repeats, two or more functional CDD-1 repeats, one or more zinc binding domains (ZBDs), binding site(s) for mooring sequences, or binding sites for auxiliary RNA binding proteins. Deaminases optionally edit viral RNA, host cell mRNA, viral DNA, host cell DNA or any combination thereof. One deaminase described herein is CEM15. CEM15 is homologous to Phorbolin or APOBEC-3G (see, for example, Accession #NP_068594). The names CEM15 and APOBEC-3G can be used interchangeably. CEM15 reduces retroviral infectivity as an RNA or DNA editing enzyme.

By "deaminating function" is meant a deamination of a nucleotide (e.g., cytidine, deoxycytidine, adenosine, or deoxyadenosine). Deaminating function is detected by measuring the amount of deaminated nucleotide, according to the methods taught herein, wherein such levels are above background levels (preferably at least 1.5 –2.5 times the background levels of the assay.)

Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on

the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Table 2 and are referred to as conservative substitutions.

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

TABLE 2: Amino Acid Substitutions

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr

Pro	Gly
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminy or histidyl residues.

Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminy and asparaginy residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, *Proteins: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

The compositions disclosed herein can be used as targets in combinatorial chemistry protocols or other screening protocols to isolate molecules that possess desired functional properties related to increasing the level of an ARPs in a subject.

As disclosed above, the disclosed compositions, such as cytidine deaminases or deoxycytidine deaminases (e.g., CEM15 and other ARPs) or Vif can be used as targets for any combinatorial technique to identify molecules or macromolecular molecules that interact with the disclosed compositions in a desired way or mimic their function. The nucleic acids,

peptides, and related molecules disclosed herein can be used as targets for the combinatorial approaches.

It is understood that when using the disclosed compositions in combinatorial techniques or screening methods, molecules, such as macromolecular molecules, will be identified that have particular desired properties such as stimulation or the target molecule's function. The molecules identified and isolated when using the disclosed compositions, such as, CEM15, other ARPs, or Vif, are also disclosed. Thus, the products produced using the combinatorial or screening approaches that involve the disclosed compositions, such as, CEM15, other ARPs, or Vif are also disclosed. Such molecules include Vif antagonists and cytidine deaminase activators.

Combinatorial chemistry includes but is not limited to all methods for isolating small molecules or macromolecules that are capable of binding either a small molecule or another macromolecule like Vif or cytidine deaminase (e.g., CEM15), typically in an iterative process. Proteins, oligonucleotides, and sugars are examples of macromolecules. For example, oligonucleotide molecules with a given function, catalytic or ligand-binding, can be isolated from a complex mixture of random oligonucleotides in what has been referred to as "*in vitro* genetics" (Szostak, TIBS 19:89, 1992). One synthesizes a large pool of molecules bearing random and defined sequences and subjects that complex mixture, for example, approximately 10¹⁵ individual sequences in 100 mg of a 100 nucleotide RNA, to some selection and enrichment process. Through repeated cycles of affinity chromatography and PCR amplification of the molecules bound to the ligand on the column, Ellington and Szostak (1990) estimated that 1 in 10¹⁰ RNA molecules folded in such a way as to bind a small molecule dyes. DNA molecules with such ligand-binding behavior have been isolated as well (Ellington and Szostak, 1992; Bock et al, 1992). Techniques aimed at similar goals exist for small organic molecules, proteins, antibodies and other macromolecules known to those of skill in the art. Screening sets of molecules for a desired activity whether based on small organic libraries, oligonucleotides, or antibodies is broadly referred to as combinatorial chemistry. Combinatorial techniques are particularly suited for defining binding interactions between molecules and for isolating molecules that have a specific binding activity, often called aptamers when the macromolecules are nucleic acids.

As used herein combinatorial methods and libraries include traditional screening methods and libraries as well as methods and libraries used in iterative processes.

The disclosed compositions can be used as targets for any molecular modeling technique to identify either the structure of the disclosed compositions or to identify potential or actual

molecules, such as small molecules, which interact in a desired way with the disclosed compositions. The compounds disclosed herein can be used as targets in any molecular modeling program or approach.

It is understood that when using the disclosed compositions in modeling techniques, molecules, such as macromolecular molecules, will be identified that have particular desired properties such as inhibition, suppression, or stimulation or the target molecule's function.

One way to isolate molecules that bind a molecule of choice is through rational design. This is achieved through structural information and computer modeling. Computer modeling technology allows visualization of the three-dimensional atomic structure of a selected molecule and the rational design of new compounds that will interact with the molecule. The three-dimensional construct typically depends on data from x-ray crystallographic analyses or NMR imaging of the selected molecule. The molecular dynamics require force field data. The computer graphics systems enable prediction of how a new compound will link to the target molecule and allow experimental manipulation of the structures of the compound and target molecule to perfect binding specificity. Prediction of what the molecule-compound interaction will be when small changes are made in one or both requires molecular mechanics software and computationally intensive computers, usually coupled with user-friendly, menu-driven interfaces between the molecular design program and the user.

Examples of molecular modeling systems are the CHARMM and QUANTA programs, Polygen Corporation, Waltham, MA. CHARMM performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modeling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen, et al., 1988 *Acta Pharmaceutica Fennica* 97, 159-166; Ripka, *New Scientist* 54-57 (June 16, 1988); McKinaly and Rossmann, 1989 *Annu. Rev. Pharmacol. Toxicol.* 29, 111-122; Perry and Davies, *QSAR: Quantitative Structure-Activity Relationships in Drug Design* pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis and Dean, 1989 *Proc. R. Soc. Lond.* 236, 125-140 and 141-162; and, with respect to a model enzyme for nucleic acid components, Askew, et al., 1989 *J. Am. Chem. Soc.* 111, 1082-1090. Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc., Pasadena, CA., Allelix, Inc, Mississauga, Ontario, Canada, and Hypercube, Inc., Cambridge, Ontario. Although these are primarily designed for application to drugs specific to

particular proteins, they can be adapted to design of molecules specifically interacting with specific regions of DNA or RNA, once that region is identified.

Although described above with reference to design and generation of compounds which can alter binding, one can also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which alter substrate binding or enzymatic activity.

Also described is a compound that is identified or designed as a result of any of the disclosed methods can be obtained (or synthesized) and tested for its biological activity, e.g., competitive stimulation of CEM15 or inhibition or suppression of viral infectivity.

Disclosed herein are computer systems and databases containing information related to APOBEC-1 Related Proteins and subjects. Since subjects will vary depending on numerous parameters including, but not limited to, race, age, weight, medical history etc., as more information is gathered on populations, the database can contain information classified by race, age, weight, medical history etc., such that one of skill in the art can assess the subject's risk of developing AIDS, the subject's susceptibility to a viral infection, the subject's ability to mount an immune response and/or the subject's responsiveness to a therapeutic agent based on information more closely associated with the subject's demographic profile.

The analysis of complex systems such as biological organisms is aided by the use of relational database systems for storing and retrieving large amounts of biological data. The advent of high-speed wide area networks and the Internet, together with the client/server based model of relational database management systems, is particularly well-suited for allowing researchers to access and meaningfully analyze large amounts of biological data given the appropriate hardware and software computing tools.

The present invention provides a computer system comprising a) a database including records comprising a plurality of reference information comprising the ARP level and associated diagnosis and therapy data; and b) a user interface capable of receiving a selection of one or more sets of information related to the subject's demographic profile.

Computer readable media include magnetically readable media, optically readable media, electronically readable media and magnetic/optical media. For example, the computer readable media may be a hard disc, a floppy disc, a magnetic tape, CD-ROM, DVD, RAM, or ROM as well as other types of other media known to those skilled in the art.

Embodiments of the present invention include systems, particularly computer systems which contain the population information described herein. As used herein, "a computer system" refers to the hardware components, software components, and data storage components used to

store and/or analyze the information of the present invention or other relevant information. The computer system preferably includes the computer readable media described above, and a processor for accessing and manipulating the data.

Preferably, the computer is a general purpose system that comprises a central processing unit (CPU), one or more data storage components for storing data, and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable.

In one particular embodiment, the computer system includes a processor connected to a bus which is connected to a main memory, preferably implemented as RAM, and one or more data storage devices, such as a hard drive and/or other computer readable media having data recorded thereon. In some embodiments, the computer system further includes one or more data retrieving devices for reading the data stored on the data storage components. The data retrieving device may represent, for example, a floppy disk drive, a compact disk drive, a magnetic tape drive, a hard disk drive, a CD-ROM drive, a DVD drive, etc. In some embodiments, the data storage component is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, etc. containing control logic and/or data recorded thereon. The computer system may advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data retrieving device. Software for accessing and processing the information of the invention (such as search tools, compare tools, modeling tools, etc.) may reside in main memory during execution.

Another aspect of the present invention is a method for determining whether a given data point from a subject differs from a point, comprising the steps of reading the information through use of a computer program which identifies differences between the test subject's information and the reference information with the computer program.

EXAMPLES

Example 1: APOBEC3G/CEM15/hA3G mRNA Levels Associates Inversely with HIV Viremia

PBMCs were obtained from consenting human subjects and cryopreserved. Prior to RNA isolation, cyropreserved PBMCs were thawed, washed with PBS, and stimulated with 1 μ g each of anti-CD3 and anti-CD28 antibodies for 18-20 hours. 2-5 x10⁶ cells were resuspended in 1 ml of TriReagent (MRC), and total cellular RNA isolated according to standard protocols. PolyA⁺ RNA was isolated using the MicroPoly (A) Purist kit (Ambion) stored in RNase-free water (Ambion) at -80°C. Purified polyA⁺ RNA was quantified by OD 260 and 280, and all

RNAs were found to have a 260/280 ratio of 1.95 or greater. hA3G gene expression was examined by using Taqman chemistry with probes and primers designed to uniquely amplify hA3G/APOBEC3G (NM_0218220). The primers used were (FWD:

5'CGCAGCCTGTGTCAGAAAAG3' (SEQ ID NO: 1, nucleotides 637-657), RVSE:

5'CCAACAGTGCTGAAATTCGTCATA3' (SEQ ID NO: 2, nucleotide 714-691) and Probe:

FAM-5'GTGCCACCATGAAGA3'-BHQ1 (SEQ ID NO: 3, nucleotide 668-682). The

following dye combinations for probe generation were used for detection and data normalization: FAM (for the genes of interest), HEX (for normalize genes, see below) and

BHQ1 (non-fluorescent quencher) and ROX. Validation experiments were performed to

determine the specificity and efficiency of the primers and probes designed to selectively amplify hA3G mRNA over closely related APOBEC3B (hA3B) and APOBEC3F (hA3F) (Wedekind et al 2003). A commercially available primer/probe combination was used to

quantify GAPDH as a normalizing control sequence for the number of cell equivalents in polyA⁺ mRNA starting material used for the quantification of hA3G mRNA. Following probe

and primer optimization, all reverse transcriptase, first strand cDNA products were diluted and

used in a 10 µl PCR reaction containing: 5 µl of ABI 2x Universal Master Mix, 1.25 µl of each forward and reverse primers (final stock concentrations ranging from 200-900nM depending on the primer set), 1 µl of probe (stock ranging from 50-200nM) and RNase/DNase free water. All reactions were run in an ABI 7900 with 1 cycle of 50 °C (2 min) followed by 95 °C (10min.) and

40 cycles of 95 °C (15 sec) followed by 60 °C (1 min). Data were collected and analyzed using Sequence Detection Software (ABI, Foster City CA), relative quantitation determined using the comparative threshold cycle (CT) method performed in Microsoft Excel (ABI Technote #2: Relative Gene Expression Quantitation).

Figure 7 is an example of this assay. Real-time PCR assays were performed using samples from a subject with HIV infection, a positive control (CEM15 control, a plasmid encoding hA3G cDNA) and a negative control (APOBEC3F, a plasmid encoding hA3F cDNA) (Fig.7a); and GAPDH from the human sample in a separate reaction (Fig.7b) as a control for cell number that were used to normalize the hA3G quantification. Each sample was tested in duplicates. These results indicated that hA3G quantification was within the reliable detection limits of the assay. Importantly, GAPDH mRNA was expressed at a similar level in each patient sample (Fig.7b).

Using this method, six HIV-uninfected and twenty-five antiretroviral naïve, chronically HIV-infected subjects, including eight LTNPs whose average viral load was 1.8×10^3 ($\pm 1.1 \times 10^3$) copies/ml, and whose average CD4 count was 755 (± 284)/µl; and seventeen progressors whose

average viral load was 1.5×10^3 ($\pm 2.5 \times 10^5$) copies/ml, and whose average CD4 count was 324 (± 208)/ μ l were studied (Table 3). HIV-1 RNA levels were quantified using the Amplicor HIV-1 Monitor assay (Roche Molecular Systems, Branchburg, NJ), which has a detection limit of 50 HIV-1 RNA copies/ml. The CD4 counts and percentages were determined using whole blood and the MultiSet program (Becton Dickinson, San Jose, CA) by flow cytometer techniques in a CLIA certified laboratory. PBMCs from these subjects were stimulated and samples were coded and sent to another lab for polyA+ mRNA extraction. The samples were recoded and sent for cDNA synthesis and real-time PCR assays. The amounts of hA3G mRNA were standardized against the GAPDH levels in each sample, and calculated as copies of mRNA/ μ g cDNA. The hA3G mRNA levels in each subject were determined, and the average values (standard deviation) in HIV-uninfected subjects were 132 (± 23) copies/ μ g cDNA, 189 (± 59) in LTNPs, and 105 (± 15) in progressors. In all HIV-infected subjects, it was 132 (± 53) (Table 3). By the Mann-Whitney test, the hA3G mRNA levels in LTNPs are significantly higher than that in progressors ($p \leq 0.001$) and HIV-uninfected controls ($p \leq 0.020$). In addition, the hA3G levels in HIV-uninfected controls is also higher than that in progressors ($p \leq 0.008$).

To determine if the augmented hA3G gene expression had any functional implications, Rank Correlation Test between hA3G mRNA levels and HIV viremia and CD4 counts in the twenty-five HIV-infected individuals was performed. There was a striking inverse correlation between hA3G mRNA levels and viral loads ($R = -0.7132$, $p \leq 0.00009$) (Fig.8a) and a highly significant positive correlation between hA3G mRNA levels and CD4 counts ($R = 0.7029$, $p \leq 0.00012$) (Fig.8b). Moreover, these correlations remain even after removing the one LTNP (#1) who has the highest CEM15 value ($R = -0.5988$, $p \leq 0.0022$ for viral load, and $R = 0.4962$, $p \leq 0.014$ for CD4 count).

Table 3. CEM15 mRNA levels in HIV-infected and -uninfected study subjects

Subject group or patient no.	Viremia (copies/ml)	CD4 count/ μ l	Yr of HIV infection	CEM15 mRNA copies/ μ g of cDNA
HIV-uninfected^a				
Mean				132
SD				23
HIV-infected				
LTNPs				
1	5.0E \pm 01	1,320	8	321
2	8.1E \pm 02	492	18	173
3	1.3E \pm 03	591	9	189
4	1.7E \pm 03	737	12	114
5	2.2E \pm 03	648	16	175
6	2.6E \pm 03	478	15	204
7	3.0E \pm 03	1,000	18	161

8	3.1E±03	775	14	176
Mean	1.8E±03	755		189
SD	1.1E±03	284		59
Progressors				
9	2.4E±03	637	2	133
10	3.0E±03	237	19	103
11	5.3E±03	247	12	98
12	5.3E±03	600	4	93
13	5.8E±03	418	NA ^b	99
14	1.6E±04	462	1	93
15	2.1E±04	582	3	121
16	2.3E±04	263	17	100
17	2.4E±04	166	NA	98
18	2.7E±04	177	6	120
19	3.7E±04	516	3	125
20	7.3E±04	209	NA	98
21	1.4E±05	599	10	94
22	2.1E±05	17	7	135
23	4.1E±05	11	11	98
24	7.5E±05	211	12	92
25	7.5E±05	157	10	89
Mean	1.5E±05	324		105
SD	2.5E±05	208		15
LTNPs + progressors				
Mean	1.0E±05	462		132
SD	2.2E±05	307		53

a *n* = 6.

b NA, not available.

Although it has been shown that hA3G contributes to the control of HIV and SIV replication in cell cultures and animal experiments (Mariani 2003; Sheehy 2002), these results are the first to demonstrate correlations between hA3G mRNA levels and HIV viral load and CD4 count, both of which are predictors of HIV disease progression in patients who have not received antiretroviral drugs or other forms of therapeutic intervention. In addition, as disclosed herein, LTNPs have significantly higher hA3G mRNA levels than did HIV-uninfected controls and the progressors, whose hA3G mRNA levels are significantly lower than that of HIV-uninfected controls.

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- Zhang, J., and D. M. Webb. 2004. Rapid evolution of primate antiviral enzyme APOBEC3G. *Hum Mol Genet* 13:1785-91.
- 20 Zheng, Y.H., Irwin, D., Kurosu, T., Tokunaga, K., Sata, T., Peterlin, B.M. (2004) Human APOBEC3F is Another Host Factor that Blocks Human Immunodeficiency Virus Type 1 Replication. *J. Virol.* 78:6073-6076.

What is claimed is:

1. A method of predicting the severity of a viral infection in a subject, comprising
 - a) acquiring a biological sample from the subject; and
 - b) measuring the level of expression of one or more APOBEC-1 related proteins in the subject, wherein a higher level of expression as compared to a reference level indicates decreased severity.
2. The method of claim 1, where in the APOBEC-1 related protein is CEM15.
3. The method of claim 1, where in the APOBEC-1 related protein is selected from the group consisting of CEM15, APOBEC-3B, APOBEC-3C, APOBEC-3F.
4. The method of claim 1, wherein the subject has a viral infection when the levels of expression are measured.
5. The method of claim 1, wherein the viral infection is a lentiviral infection.
6. The method of claim 1, wherein the lentiviral infection is an HIV-1 infection.
7. The method of claim 1, wherein the decreased severity comprises an increased longevity in the subject as compared to a control.
8. The method of claim 1, wherein the decreased severity comprises a longer asymptomatic period in the subject as compared to a control
9. The method of claim 8, wherein the asymptomatic period is one year or longer than the control asymptomatic period.
10. The method of claim 1, wherein the decreased severity comprises high CD4 counts as compared to a control.
11. The method of claim 1, wherein the decreased severity comprises lower HIV viremia levels as compared to a control.
12. The method of claim 1, wherein expression is measured by detecting mRNA levels of APOBEC-1 related protein.
13. The method of claim 12, wherein the mRNA levels are detected by PCR.
14. The method of claim 13, wherein the PCR is real time PCR (RT-PCR).
15. The method of claim 1, wherein the expression is measured by detecting protein levels of APOBEC-1 related proteins.
16. The method of claim 15, wherein CEM15 protein levels are detected using Western blotting.
17. The method of claim 15, wherein CEM15 protein levels are detected using ELISA.
18. The method of claim 1, wherein the sample is a blood sample.

19. The method of claim 1, wherein the sample is a cellular extract.
20. The method of claim 1, wherein the sample is a tissue extract.
21. A method of predicting whether a subject is or will be a long term nonprogressor (LTNP) when infected with a virus, comprising
 - a) acquiring a biological sample from the subject; and
 - b) measuring the level of expression of one or more APOBEC-1 related proteins in the subject, wherein a higher level of expression as compared to a reference level indicates the subject is a potential LTNP.
22. The method of claim 21, wherein the APOBEC-1 related protein is CEM15.
23. The method of claim 21, wherein the APOBEC-1 related protein is selected from the group consisting of CEM15, APOBEC-3B, APOBEC-3C, APOBEC-3F.
24. The method of claim 21, wherein the subject has a viral infection when the levels of expression are measured.
25. The method of claim 21, wherein expression is measured by detecting mRNA for APOBEC-1 related proteins.
26. The method of claim 25, wherein the mRNA levels are detected by PCR.
27. The method of claim 26, wherein the PCR is real time PCR (RT-PCR).
28. The method of claim 21, wherein the expression is measured by detecting protein levels of the APOBEC-1 related proteins.
29. The method of claim 28, wherein the protein levels of the APOBEC-1 related proteins are detected using Western blotting.
30. The method of claim 28, wherein CEM15 protein levels are detected using ELISA.
31. The method of claim 21, wherein the sample is a blood sample.
32. A method of optimizing antiviral therapy in a subject with a viral infection comprising:
 - a) acquiring a biological sample from the subject;
 - b) detecting the level of expression of one or more APOBEC-1 related proteins in the sample; and
 - c) adjusting the antiviral therapy according to the levels APOBEC-1 related proteins, thereby optimizing the viral therapy.
33. The method of claim 32, wherein the antiviral therapy is selected from the group consisting of a nucleoside reverse transcriptase inhibitor, non-nucleoside reverse transcriptase inhibitor, nucleotide reverse transcriptase inhibitor, protease inhibitor, fusion inhibitor, and an integrase inhibitor.

34. The method of claim 32, wherein the antiviral therapy is reduced when the expression levels of APOBEC-1 related proteins is high as compared to a reference level.
35. The method of claim 32, wherein the antiviral therapy is increased when the expression levels of APOBEC-1 related proteins is low as compared to a reference level.
36. The method of claim 32, where in the APOBEC-1 related protein is CEM15.
37. The method of claim 32, where in the APOBEC-1 related protein is selected from the group consisting of CEM15, APOBEC-3B, APOBEC-3C, APOBEC-3F.
38. A method of predicting the level of CD4 cells in a subject, comprising
 - a) acquiring a biological sample from the subject;
 - b) detecting the level of CEM15 expression in the subject, the level of CEM15 correlating with the level of CD4 cells.
39. A method of monitoring effectiveness of an antiviral agent in a subject, comprising:
 - a) detecting expression levels of one or more APOBEC-1 related proteins in a first biological sample from the subject prior to administration of the agent; and
 - b) detecting expression levels of one or more APOBEC-1 related proteins in a second biological sample from the subject after administration of the agent, an increase in expression levels of the APOBEC-1 related proteins in the second sample as compared to the first sample indicating effectiveness of the antiviral agent.
40. The method of claim 39, wherein the APOBEC-1 related protein is CEM15.
41. The method of claim 40, wherein the agent targets a Vif/CEM15 interaction.
42. The method of claim 41, wherein the agent is a Vif antagonist.
43. The method of claim 39, where in the APOBEC-1 related protein is selected from the group consisting of CEM15, APOBEC-3B, APOBEC-3C, APOBEC-3F.
44. The method of claim 39, wherein the agent is selected from the group consisting of nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors, protease inhibitor, and fusion inhibitors.
45. The method of claim 39, wherein the subject has a viral infection when the levels of expression are measured.
46. The method of claim 45, wherein the subject is infected with a lentivirus.
47. The method of claim 45, wherein the lentivirus is HIV-1.
48. A method of screening for an antiviral agent, comprising
 - a) administering to a subject with a viral infection an agent to be screened; and

- b) detecting expression levels of one or more APOBEC-1 related proteins in a biological sample from the subject, an increased expression level indicating an antiviral agent.
49. The method of claim 48, wherein the APOBEC-1 related protein is CEM15.
 50. The method of claim 48, wherein the APOBEC-1 related protein is selected from the group consisting of CEM15, APOBEC-3B, APOBEC-3C, APOBEC-3F.
 51. An isolated nucleic acid sequence comprising a sequence at least 80% identical to SEQ ID NO: 1.
 52. An isolated nucleic acid sequence comprising the nucleotide sequence of SEQ ID NO: 1, or variants or fragments thereof, wherein the variant or fragment comprises a specific CEM15 primer.
 53. An isolated nucleic acid sequence comprising at least five consecutive nucleotides of SEQ ID NO: 1, wherein the nucleic acid sequence comprises a specific CEM15 primer.
 54. An isolated nucleic acid sequence comprising the sequence of SEQ ID NO: 1.
 55. An isolated nucleic acid sequence comprising a sequence at least 80% identical to SEQ ID NO: 2.
 56. An isolated nucleic acid sequence comprising the nucleotide sequence of SEQ ID NO: 2, or variants or fragments thereof, wherein the variant or fragment comprises a specific CEM15 primer.
 57. An isolated nucleic acid sequence comprising at least five consecutive nucleotides of SEQ ID NO: 2, wherein the nucleic acid sequence comprises a specific CEM15 primer.
 58. An isolated nucleic acid sequence comprising SDEQ ID NO:2.
 59. An isolated nucleic acid sequence comprising a sequence at least 80% identical to SEQ ID NO: 3.
 60. An isolated nucleic acid sequence comprising the nucleotide sequence of SEQ ID NO: 3, or variants or fragments thereof, wherein the variant or fragment comprises a specific CEM15 probe.
 61. An isolated nucleic acid sequence comprising at least five consecutive nucleotides of SEQ ID NO: 3, wherein the nucleic acid sequence comprises a specific CEM15 probe.
 62. An isolated nucleic acid sequence comprising at least five consecutive nucleotides of SEQ ID NO: 15, wherein the nucleic acid sequence comprises a specific APOBEC-3B primer.

63. An isolated nucleic acid sequence comprising at least five consecutive nucleotides of SEQ ID NO: 16, wherein the nucleic acid sequence comprises a specific APOBEC-3B primer.
64. An isolated nucleic acid sequence comprising at least five consecutive nucleotides of SEQ ID NO: 17, wherein the nucleic acid sequence comprises a specific APOBEC-3C primer.
65. An isolated nucleic acid sequence comprising at least five consecutive nucleotides of SEQ ID NO: 18, wherein the nucleic acid sequence comprises a specific APOBEC-3F primer.
66. An isolated nucleic acid sequence comprising at least five consecutive nucleotides of SEQ ID NO: 19, wherein the nucleic acid sequence comprises a specific APOBEC-3F primer.
67. A method of treating a subject infected with a virus with an appropriate antiviral agent, comprising the steps of:
 - a) identifying a population of subjects with a given range of APOBEC-1 related protein levels;
 - b) determining which antiviral agent is most effective at the given range of APOBEC-1 related protein levels; and
 - c) administering an appropriate antiviral agent to the subject in need thereof.
68. The method of claim 67, wherein the APOBEC-1 related protein is selected from the group consisting of CEM15, APOBEC-3B, APOBEC-3C, APOBEC-3F.
69. The method of claim 67, wherein the agent targets a Vif/CEM15 interaction.
70. The method of claim 69, wherein the agent is a Vif antagonist.
71. The method of claim 67, wherein the agent is selected from the group consisting of nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors, protease inhibitor, and fusion inhibitors.
72. The method of claim 67, wherein the subject has a viral infection when the levels of expression are measured.
73. The method of claim 72, wherein the subject is infected with a lentivirus.
74. The method of claim 72, wherein the lentivirus is HIV-1.

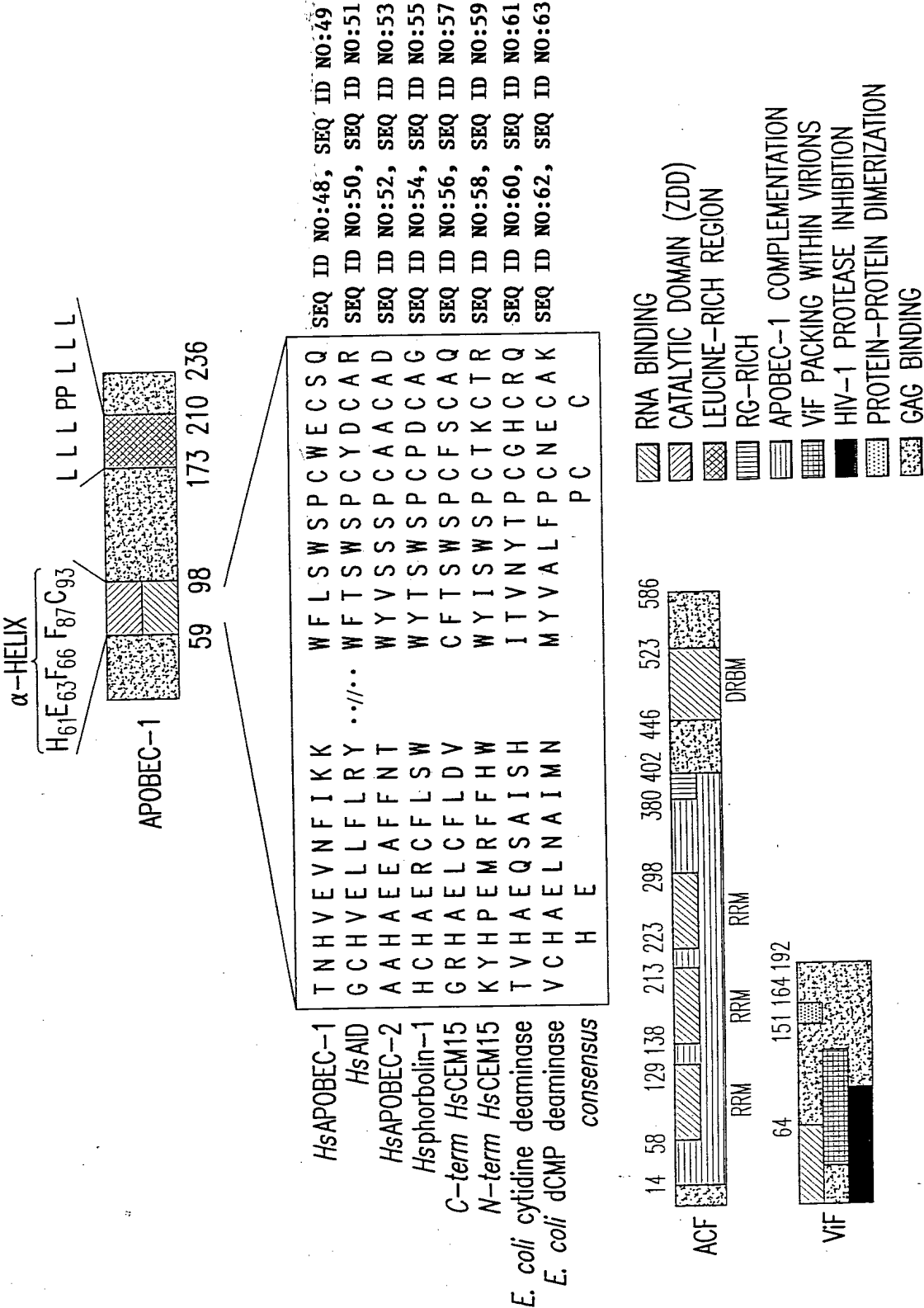
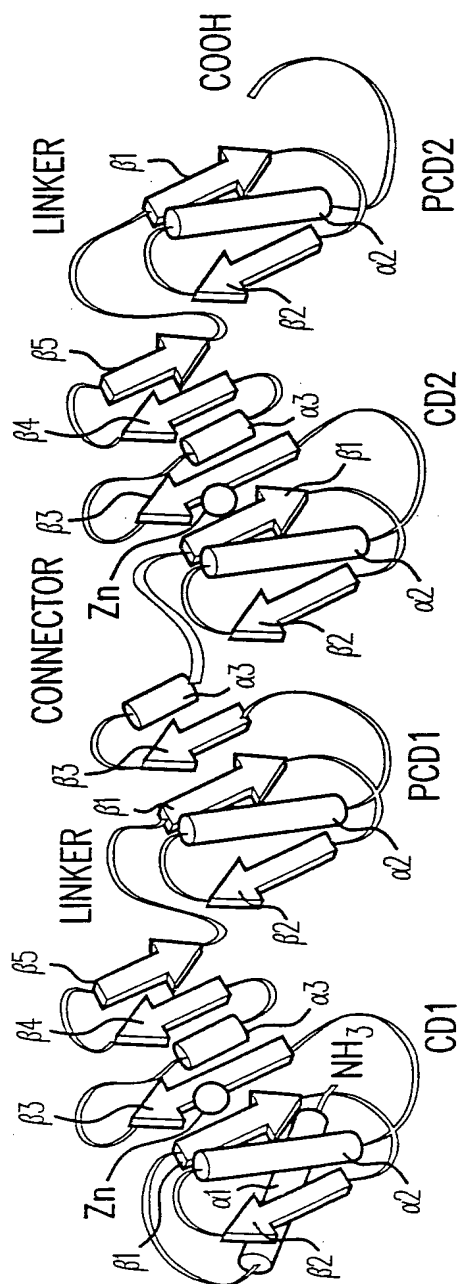


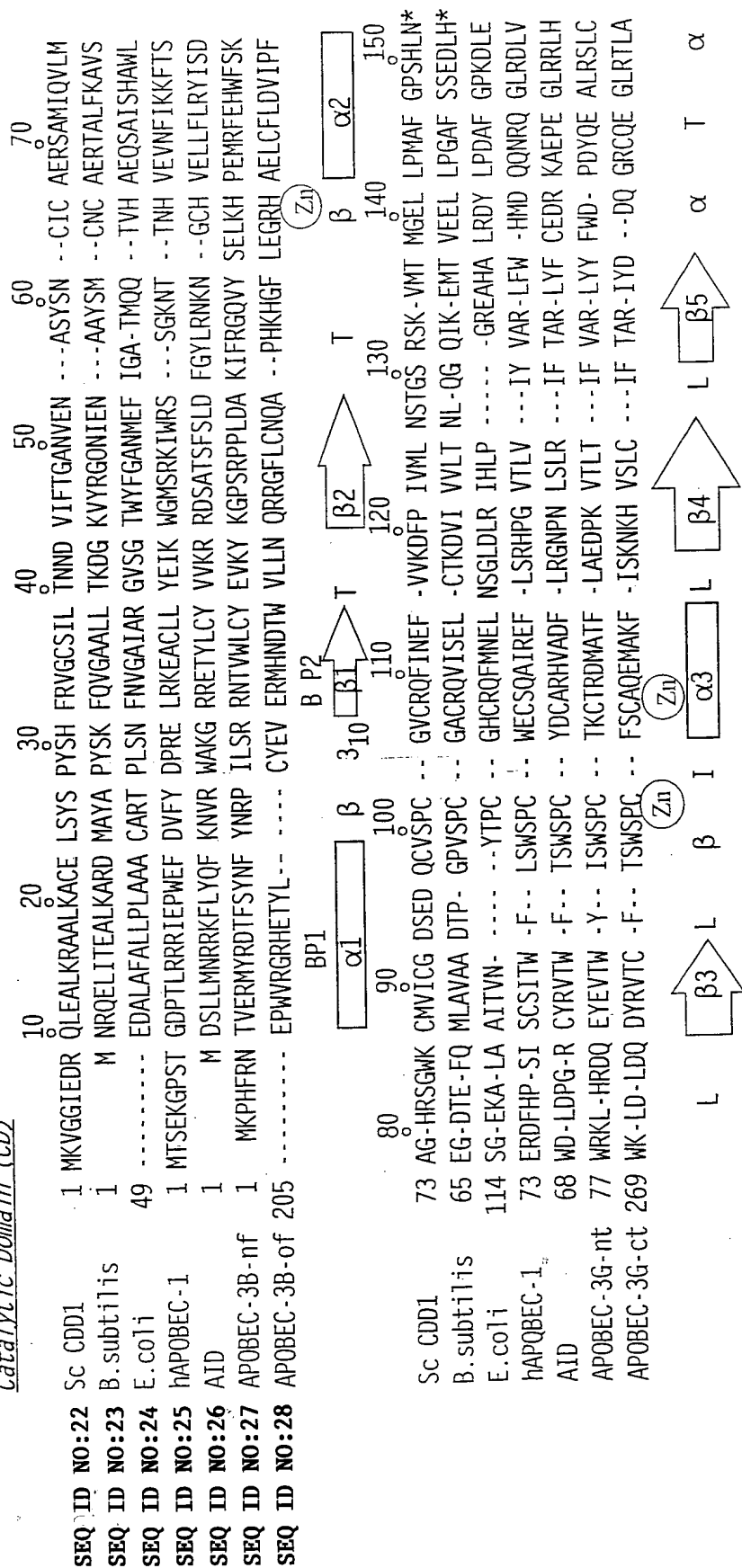
FIG. 1

[illegible]

APOBEC-3B-LIKE


$$\text{RX}(15,18) \text{ [C/H]XEX}_2[\text{F/A}][\text{L/M}]\text{X}(15,16)[\text{L/V}]\text{X}(2,9)[\text{S/T}]\text{PCX}(2,4)\text{CX}_2[\text{L/V/M}]\text{X}_2[\text{F/L}]\text{X}(6,7)[\text{L/V}]\text{X}[\text{LM}]\text{X}(2,8)[\text{K/R}]\text{X}(14,16)\text{L}$$

Catalytic Domain (CD)



Linker Polypeptide

SEQ ID NO:29	E. coli	172°	IKPLLMEQDQHGVALTG
SEQ ID NO:30	hAPOBEC-1	136°	NSGVITI
SEQ ID NO:31	AID	131°	RAGVQIAI
SEQ ID NO:32	APOBEC-3G-nt	141°	KRDGPRATMKI
SEQ ID NO:33	APOBEC-3G-ct	330°	EAGAKISI

FIG. 2B-1

Pseudocatalytic Domain (PCD)

SEQ ID NO:34
SEQ ID NO:35
SEQ ID NO:36
SEQ ID NO:37
SEQ ID NO:38
SEQ ID NO:39
SEQ ID NO:40
SEQ ID NO:41

160 170 180 190 200 210 220 230
Sc CDD1 1 MKVGGIEDR QLEALKRAALKACE LSYS PYSH FRVGCISIL TNND VIFTGANVEN ASYSN CIC AERSAMIQVLM AG-HR-SGHWK
B.subtilis 1 -----M NRQELITEALKARD MAYA PYSK FQVGAALL TKDG KVRGCGNIEN AAYSM CNC AERTALFKAVS EG-DT--EFQ
E.coli 189 ----- --DALSQAAIAAAN RSHM PYSK SPGSVALE CKDG RIFSGSYAEN AAFNP TLP PLQGALILLNL KG-YDYPDIQ
hAPOBEC-1-nt 1 MTSEKGPST GDPTLRRRIEPWEF DVFY DPRE LRKEACLL YEIK WGMRSKIWRS SGKNT TNH VEVNFIKKFTS ERDF--HPSI
hAPOBEC-1-cf 144 ----- MRASEYYH CWRN FVNYPPGDEA H--WP QYP PLWMMLYALEL HC-IILSLPP
AID 139 ----- MTFKDYFY CWNT FVENHERTEK A--WE GLH ENSVRLTRQLR RI-L-L-PL-
APOBEC-3G-nt 152 ----- MNYDEFQH CWSK FVYSQRELFE P--WN NLP KYVILLHIMLG EI-LRHSMNP
APOBEC-3G-ct 338 ----- MTYSEFKH CWDT FVDHQGCPFQ P--WD GLD EHSQDLSGRRL AI-LQNQGEN*



240 250 260 270 280 290 300 310
Sc CDD1 80 CMVICG DSEDQCVS ----- PC GVCRRQFINEF VKDFFP IVML NSTGS RSKVMT MGEL LPIAF GPSHLN*
B.subtilis 72 MLAVAA DTP-GPVS ----- PC GACRQVISEL CTKDVI VLT NL-QG QIKEMT VEEL LPGAF SSEDHL*
E.coli 259 RAVLAE ----- KADAPLIQW -- DATSATLKAL GC--HS IDRV LL-A*
hAPOBEC-1-nt 81 SCSITW -F--LSWS ----- PC WECSQAIRSF LSRHPG VILV -I-Y- VARLFW -HND QQNRQ GLRDLV*
hAPOBEC-1-ot 192 CLKISR ----- R--WQNHLT FF ELHLQNCYQ T---IP PHIL LAT-L IHPSVA WR*
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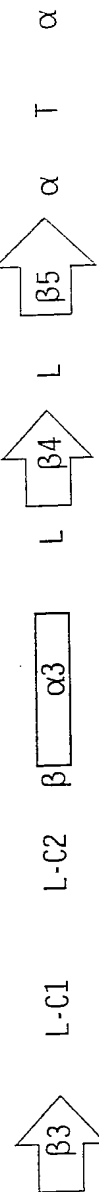


FIG. 2B-2

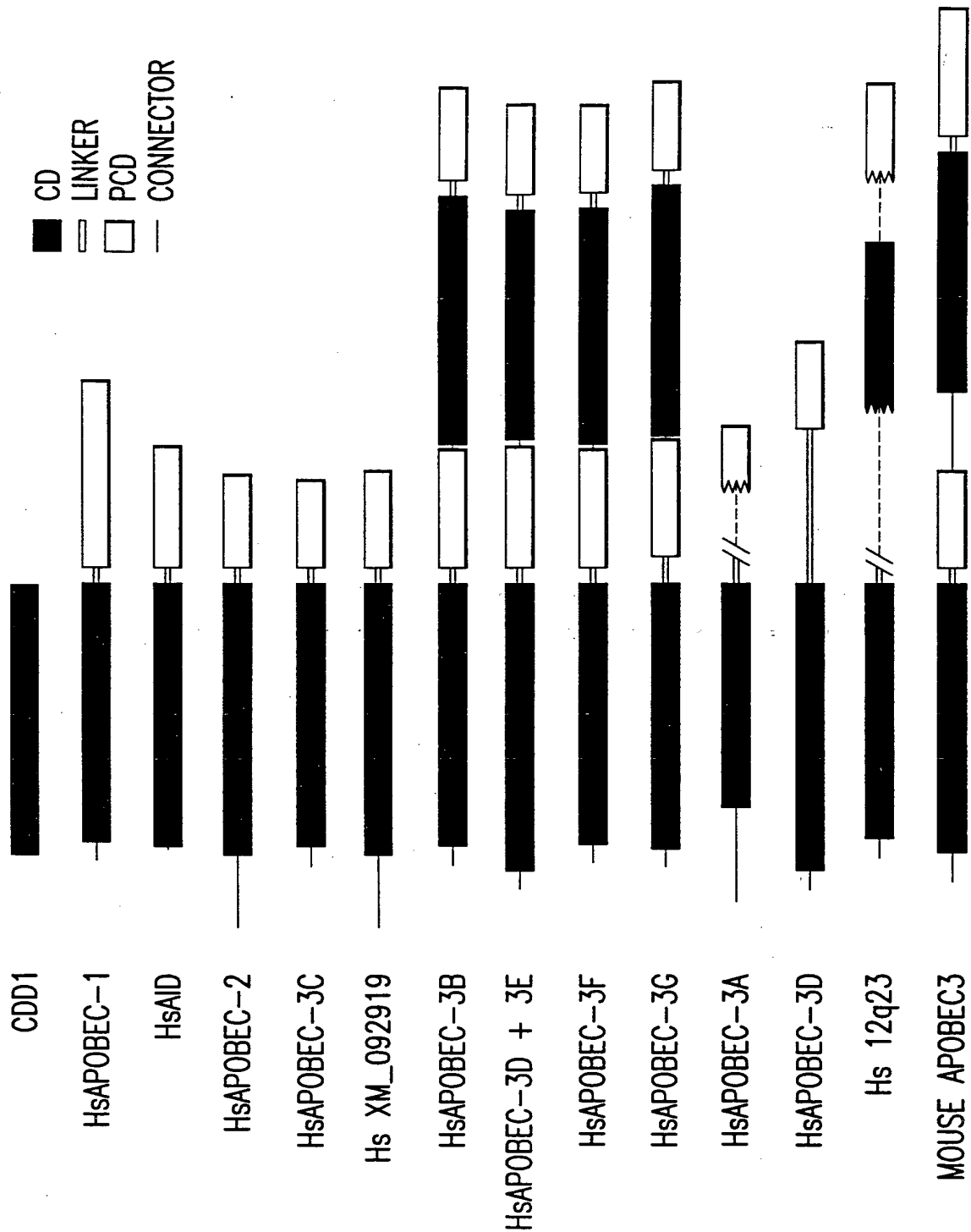


FIG. 2C

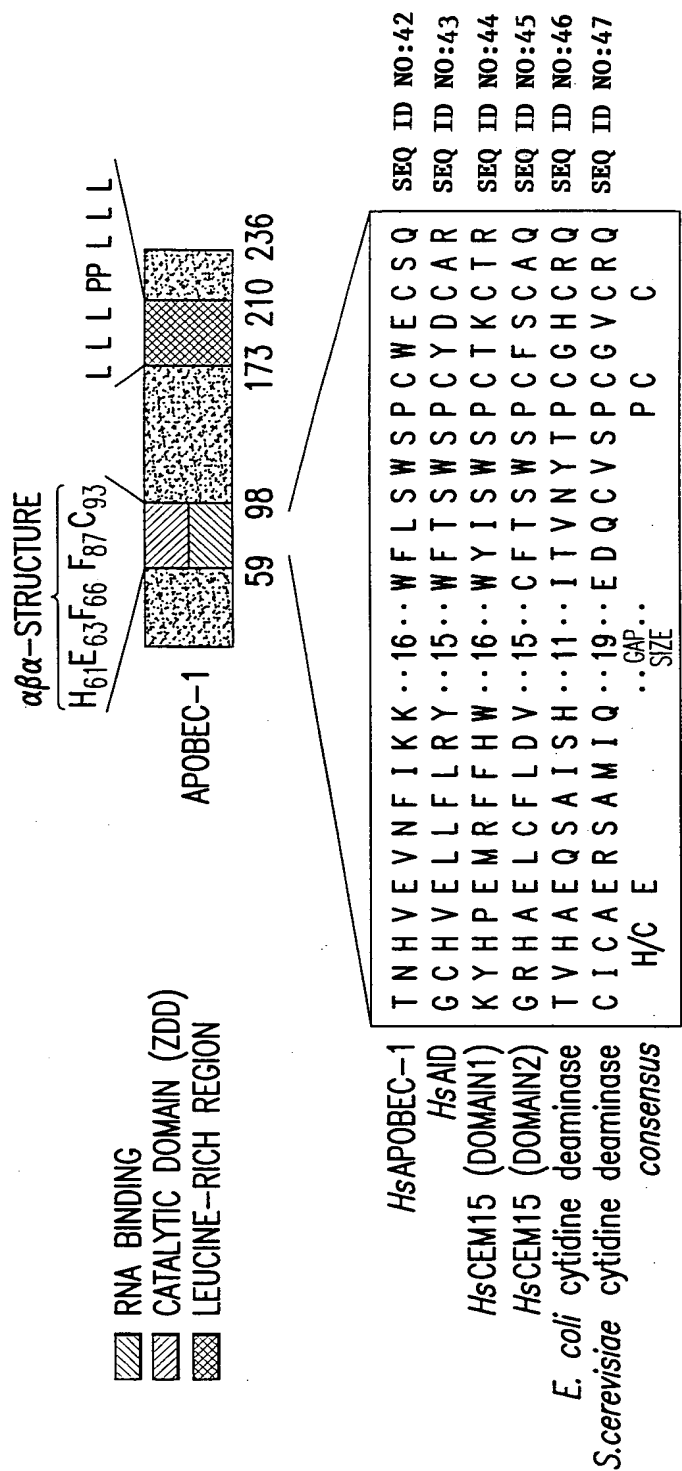


FIG. 3

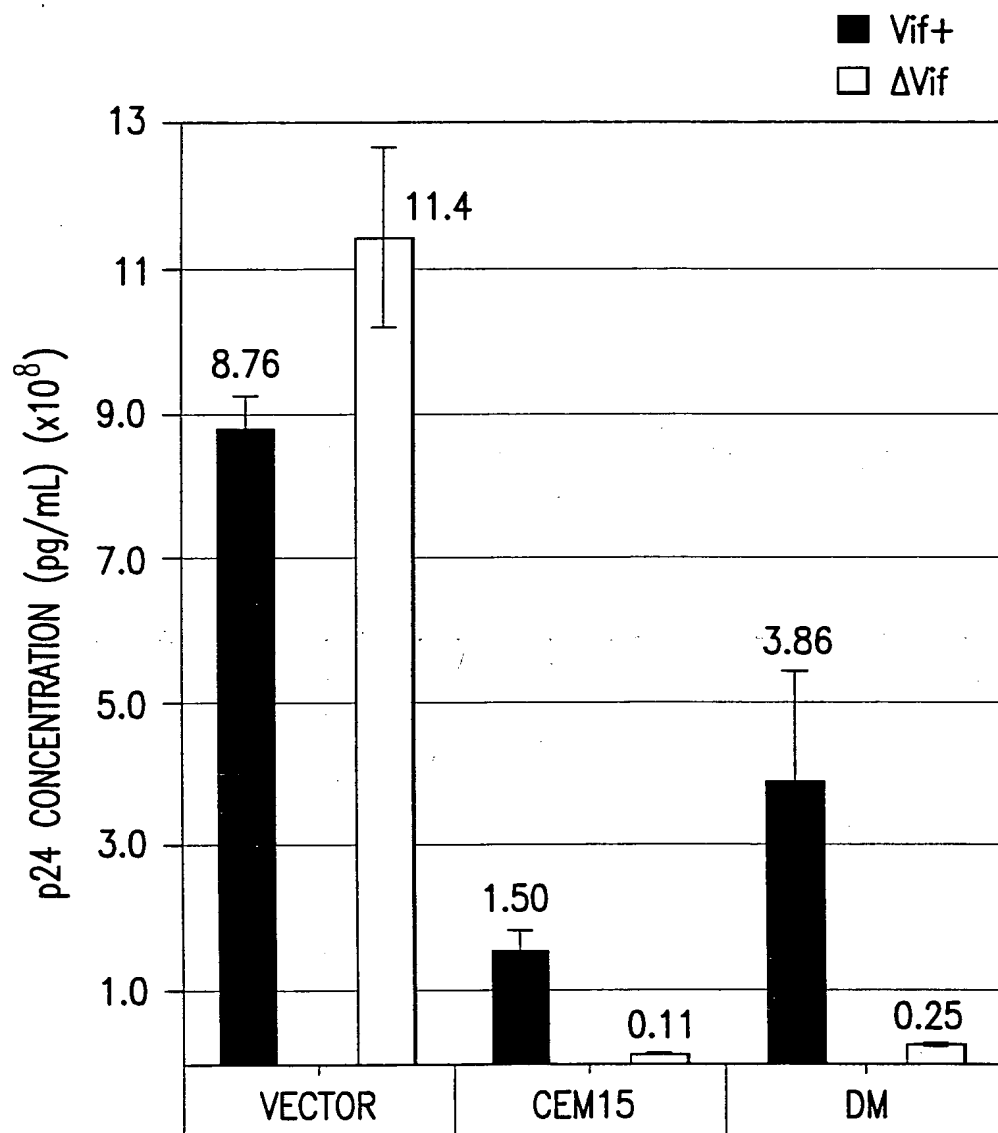


FIG. 4

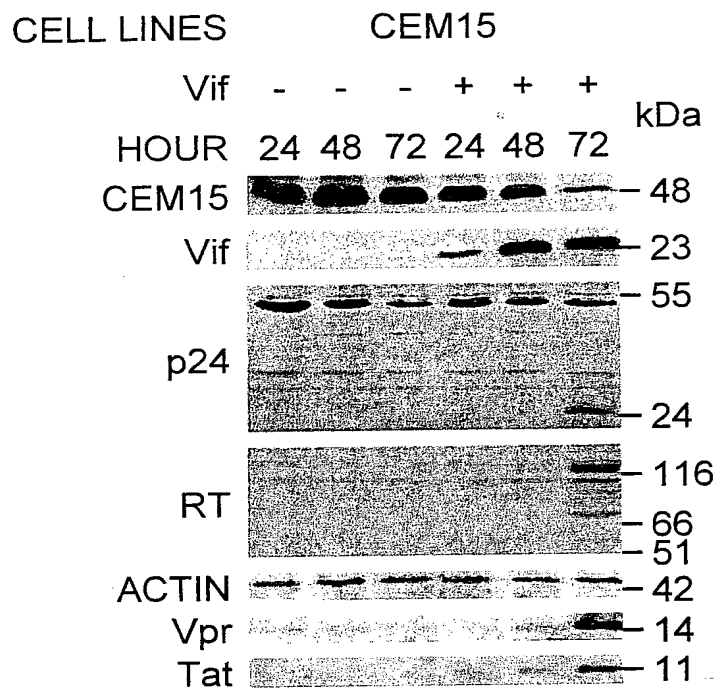


FIG. 5A

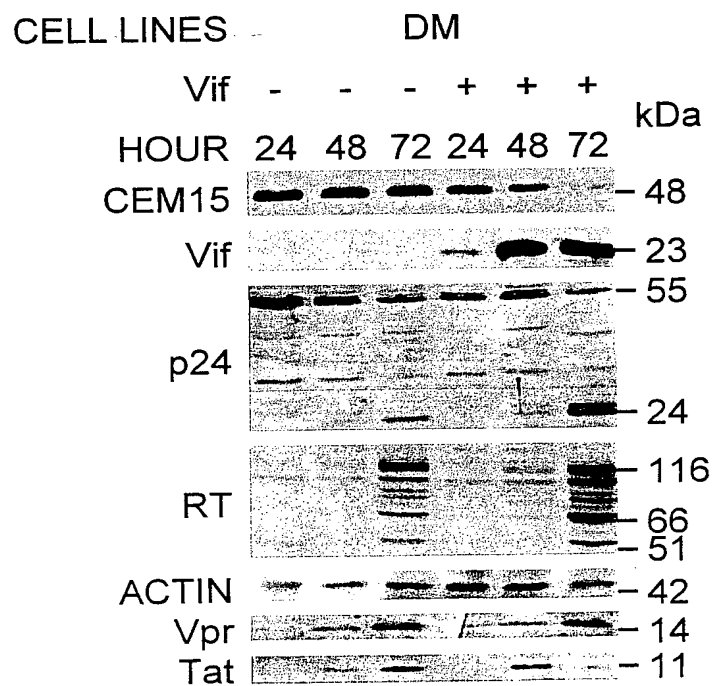


FIG. 5B

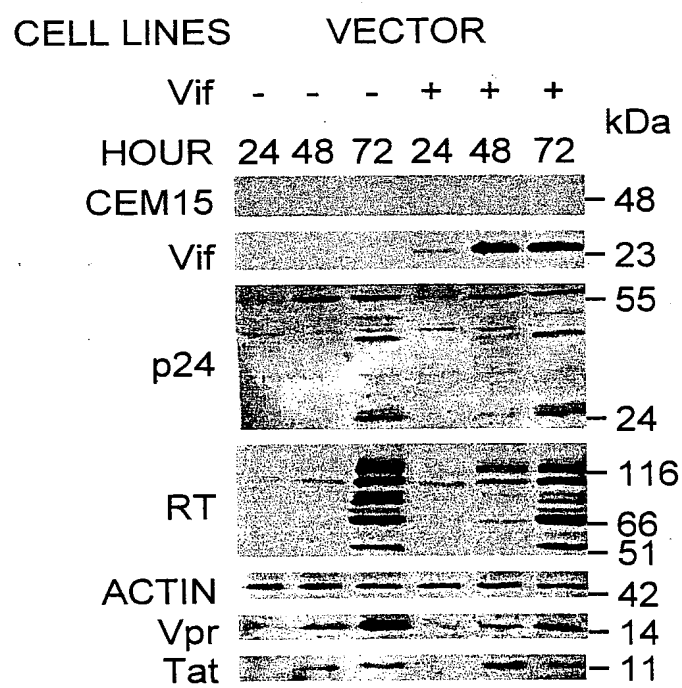


FIG. 5C

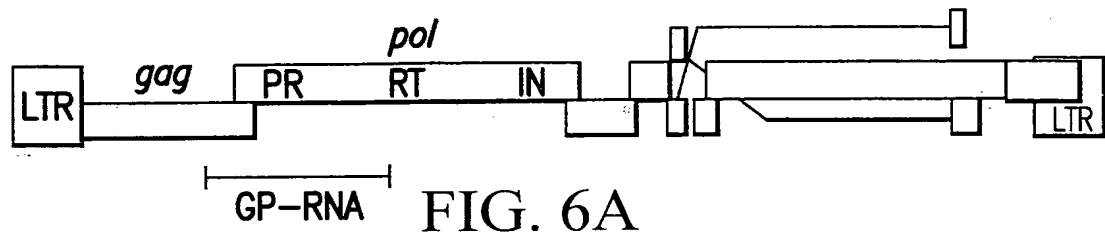


FIG. 6B

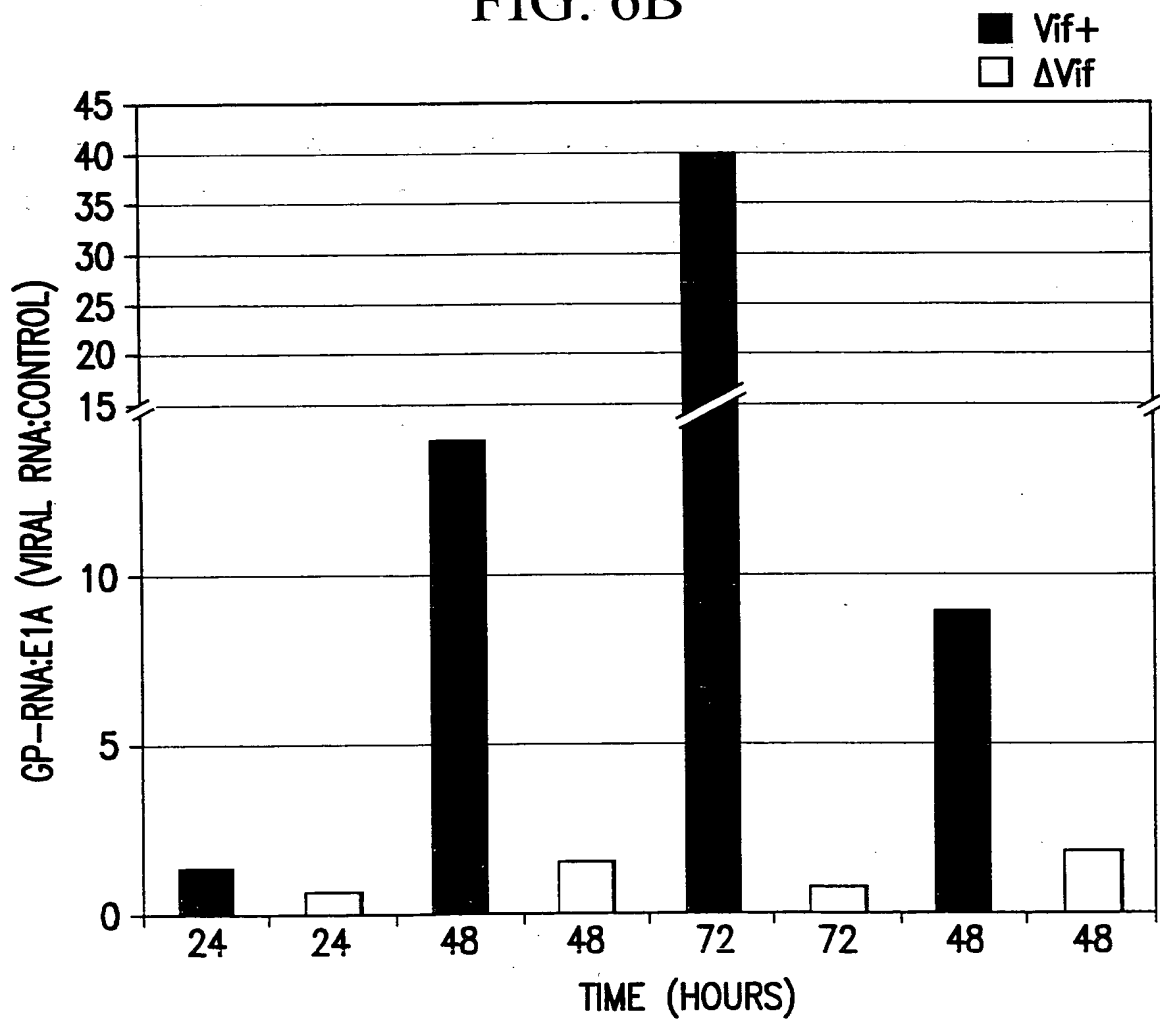


FIG. 6C

CEM15

DM

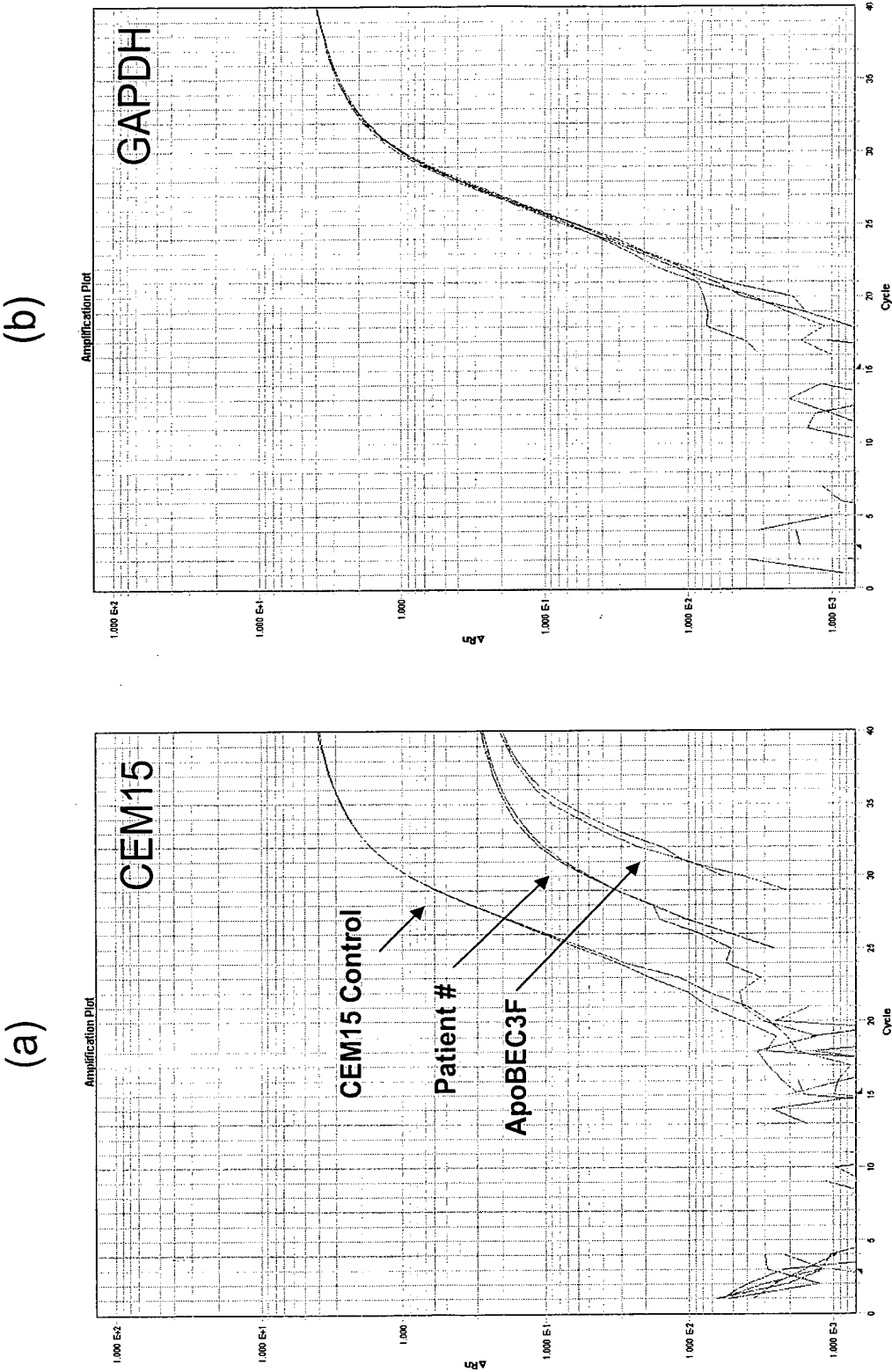


FIG. 7

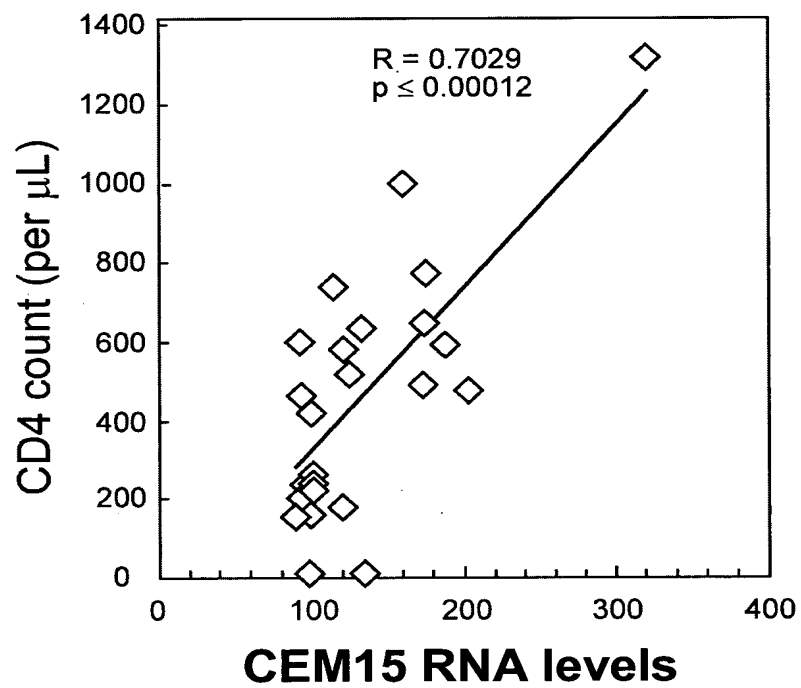
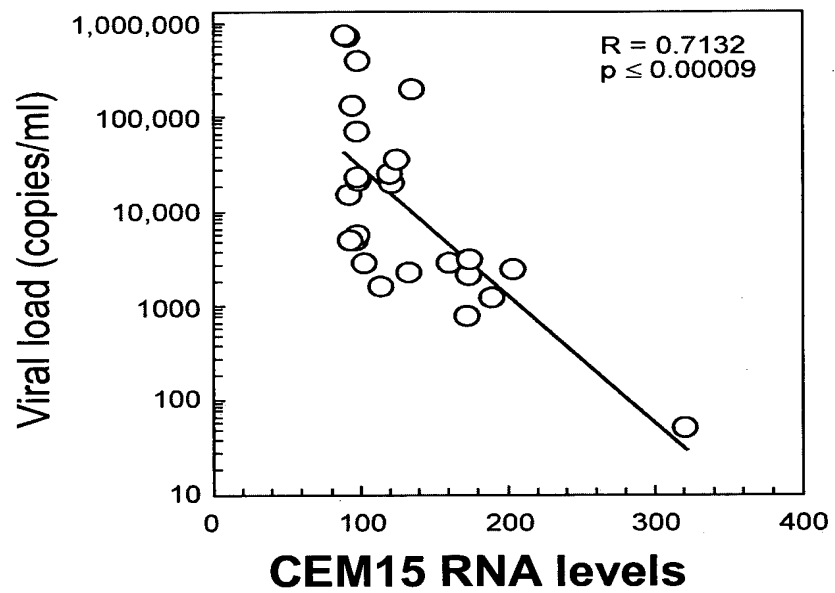


FIG. 8

SEQUENCE LISTING

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SMITH, Harold C., et al.

<120> METHODS AND COMPOSITIONS RELATED TO
HEIGHTENED APOBEC-1 RELATED PROTEIN (ARP) EXPRESSION

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<140> New PCT

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Val Trp Leu Cys Tyr Glu Val Lys Thr Lys Gly Pro Ser Arg Pro Pro
          35           40           45
Leu Asp Ala Lys Ile Phe Arg Gly Gln Val Tyr Ser Glu Leu Lys Tyr
          50           55           60
His Pro Glu Met Arg Phe Phe His Trp Phe Ser Lys Trp Arg Lys Leu
65           70           75           80
His Arg Asp Gln Glu Tyr Glu Val Thr Trp Tyr Ile Ser Trp Ser Pro
          85           90           95
Cys Thr Lys Cys Thr Arg Asp Met Ala Thr Phe Leu Ala Glu Asp Pro
          100          105          110
Lys Val Thr Leu Thr Ile Phe Val Ala Arg Leu Tyr Tyr Phe Trp Asp
          115          120          125
Pro Asp Tyr Gln Glu Ala Leu Arg Ser Leu Cys Gln Lys Arg Asp Gly
          130          135          140
Pro Arg Ala Thr Met Lys Ile Met Asn Tyr Asp Glu Phe Gln His Cys
145          150          155          160
Trp Ser Lys Phe Val Tyr Ser Gln Arg Glu Leu Phe Glu Pro Trp Asn
          165          170          175
Asn Leu Pro Lys Tyr Tyr Ile Leu Leu His Ile Met Leu Gly Glu Ile
          180          185          190
Leu Arg His Ser Met Asp Pro Pro Thr Phe Thr Phe Asn Phe Asn Asn
          195          200          205
Glu Pro Trp Val Arg Gly Arg His Glu Thr Tyr Leu Cys Tyr Glu Val
          210          215          220
Glu Arg Met His Asn Asp Thr Trp Val Leu Leu Asn Gln Arg Arg Gly
225          230          235          240
Phe Leu Cys Asn Gln Ala Pro His Lys His Gly Phe Leu Glu Gly Arg
          245          250          255
His Ala Glu Leu Cys Phe Leu Asp Val Ile Pro Phe Trp Lys Leu Asp
          260          265          270
Leu Asp Gln Asp Tyr Arg Val Thr Cys Phe Thr Ser Trp Ser Pro Cys
          275          280          285
Phe Ser Cys Ala Gln Glu Met Ala Lys Phe Ile Ser Lys Asn Lys His
          290          295          300
Val Ser Leu Cys Ile Phe Thr Ala Arg Ile Tyr Asp Asp Gln Gly Arg
305          310          315          320
Cys Gln Glu Gly Leu Arg Thr Leu Ala Glu Ala Gly Ala Lys Ile Ser
          325          330          335
Ile Met Thr Tyr Ser Glu Phe Lys His Cys Trp Asp Thr Phe Val Asp
          340          345          350
His Gln Gly Cys Pro Phe Gln Pro Trp Asp Gly Leu Asp Glu His Ser
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Phe Ser Tyr Asn Phe Tyr Asn Arg Pro Ile Leu Ser Arg Arg Asn Thr
20          25          30
Val Trp Leu Cys Tyr Glu Val Lys Thr Lys Gly Pro Ser Arg Pro Pro
35          40          45
Leu Asp Ala Lys Ile Phe Arg Gly Gln Val Tyr Ser Glu Leu Lys Tyr
50          55          60
His Pro Glu Met Arg Phe Phe His Trp Phe Ser Lys Trp Arg Lys Leu
65          70          75          80
His Arg Asp Gln Glu Tyr Glu Val Thr Trp Tyr Ile Ser Trp Ser Pro
85          90          95
Cys Thr Lys Cys Thr Arg Asp Met Ala Thr Phe Leu Ala Glu Asp Pro
100         105         110
Lys Val Thr Leu Thr Ile Phe Val Ala Arg Leu Tyr Tyr Phe Trp Asp
115         120         125

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4/24

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 130 135 140
 Pro Arg Ala Thr Met Lys Ile Met Asn Tyr Asp Glu Phe Gln His Cys
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 165 170 175
 Asn Leu Pro Lys Tyr Tyr Ile Leu Leu His Ile Met Leu Gly Glu Ile
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 210 215 220
 Glu Arg Met His Asn Asp Thr Trp Val Leu Leu Asn Gln Arg Arg Gly
 225 230 235 240
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 Ile Met Thr Tyr Ser Glu Phe Lys His Cys Trp Asp Thr Phe Val Asp
 340 345 350
 His Gln Gly Cys Pro Phe Gln Pro Trp Asp Gly Leu Asp Glu His Ser
 355 360 365
 Gln Asp Leu Ser Gly Arg Leu Arg Ala Ile Leu Gln Asn Gln Glu Asn
 370 375 380

<210> 7

<211> 1155

<212> DNA

<213> Artificial Sequence

<220>

 <223> Description of Artificial Sequences; note =
 synthetic construct

<400> 7

atgaagcctc	acttcagaaa	cacagtggag	cgaatgtatc	gagacacatt	ctcctacaac	60
ttttataata	gacccatcct	ttctcgtcgg	aataccgtct	ggctgtgcta	cgaagtgaac	120
acaaagggtc	cctcaaggcc	ccctttggac	gcaaagatct	ttcgaggcca	ggtgtattcc	180
gaacttaagt	accacccaga	gatgagattc	ttccactggg	tcagcaagtg	gaggaagctg	240
catcgtgacc	aggagtatga	ggtcacctgg	tacatatcct	ggagcccctg	cacaaagtgt	300
acaagggata	tgggcacggt	cctggccgag	gacccgaagg	ttaccctgac	catcttcggt	360
gcccgcctct	actactttctg	ggacccagat	taccaggagg	cgcttcgcag	cctgtgtcag	420
aaaagagacg	gtccgcgtgc	caccatgaag	atcatgaatt	atgacgaatt	tcagcactgt	480
tgaggaagct	tcgtgtacag	ccaaagagag	ctatttgagc	cttggaataa	tctgcctaaa	540
tattatatat	tactgcacat	catgctgggg	gagattctca	gacactcgat	ggatccaccc	600
acattcactt	tcaacttttaa	caatgaacct	tggtgcagag	gacggcatga	gacttacctg	660
tggtatgagg	tgagagcgc	gcacaatgac	acctgggtcc	tgctgaacca	gcgcaggggc	720
tttctatgca	accaggctcc	acataaacac	ggtttccttg	aaggccgcca	tgcagagctg	780
tgcttcctgg	acgtgtattcc	cttttggaag	ctggacctgg	accaggacta	cagggttacc	840
tgcttcacct	cctggagccc	ctgcttcagc	tgtgcccagg	aaatggctaa	attcattttca	900
aaaaacaaac	acgtgagcct	gtgcatcttc	actgcccgc	tctatgatga	tcaaggaaga	960
tgctcaggagg	ggctgcgcac	cctggccgag	gctggggcca	aaattttcaat	aatgacatac	1020
agtgaattta	agcactgctg	ggacaccttt	gtggaccacc	agggatgtcc	cttcagcccc	1080

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tgggatggac tagatgagca cagccaagac ctgagtggga ggctgcgggc cattctccag 1140
aatcaggaaa actga 1155

<210> 8

<211> 382

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 8

Met	Asn	Pro	Gln	Ile	Arg	Asn	Pro	Met	Glu	Arg	Met	Tyr	Arg	Asp	Thr
1				5					10					15	
Phe	Tyr	Asp	Asn	Phe	Glu	Asn	Glu	Pro	Ile	Leu	Tyr	Gly	Arg	Ser	Tyr
			20					25					30		
Thr	Trp	Leu	Cys	Tyr	Glu	Val	Lys	Ile	Lys	Arg	Gly	Arg	Ser	Asn	Leu
		35					40					45			
Leu	Trp	Asp	Thr	Gly	Val	Phe	Arg	Gly	Gln	Val	Tyr	Phe	Lys	Pro	Gln
	50					55					60				
Tyr	His	Ala	Glu	Met	Cys	Phe	Leu	Ser	Trp	Phe	Cys	Gly	Asn	Gln	Leu
65					70					75				80	
Pro	Ala	Tyr	Lys	Cys	Phe	Gln	Ile	Thr	Trp	Phe	Val	Ser	Trp	Thr	Pro
				85					90					95	
Cys	Pro	Asp	Cys	Val	Ala	Lys	Leu	Ala	Glu	Phe	Leu	Ser	Glu	His	Pro
			100					105					110		
Asn	Val	Thr	Leu	Thr	Ile	Ser	Ala	Ala	Arg	Leu	Tyr	Tyr	Tyr	Trp	Glu
		115					120					125			
Arg	Asp	Tyr	Arg	Arg	Ala	Leu	Cys	Arg	Leu	Ser	Gln	Ala	Gly	Ala	Arg
	130					135					140				
Val	Thr	Ile	Met	Asp	Tyr	Glu	Glu	Phe	Ala	Tyr	Cys	Trp	Glu	Asn	Phe
145					150				155					160	
Val	Tyr	Asn	Glu	Gly	Gln	Gln	Phe	Met	Pro	Trp	Tyr	Lys	Phe	Asp	Glu
				165					170					175	
Asn	Tyr	Ala	Phe	Leu	His	Arg	Thr	Leu	Lys	Glu	Ile	Leu	Arg	Tyr	Leu
			180					185					190		
Met	Asp	Pro	Asp	Thr	Phe	Thr	Phe	Asn	Phe	Asn	Asn	Asp	Pro	Leu	Val
		195					200					205			
Leu	Arg	Arg	Arg	Gln	Thr	Tyr	Leu	Cys	Tyr	Glu	Val	Glu	Arg	Leu	Asp
	210					215						220			
Asn	Gly	Thr	Trp	Val	Leu	Met	Asp	Gln	His	Met	Gly	Phe	Leu	Cys	Asn
225					230					235				240	
Glu	Ala	Lys	Asn	Leu	Cys	Gly	Phe	Tyr	Gly	Arg	His	Ala	Glu	Leu	
			245						250				255		
Arg	Phe	Leu	Asp	Leu	Val	Pro	Ser	Leu	Gln	Leu	Asp	Pro	Ala	Gln	Ile
			260					265					270		
Tyr	Arg	Val	Thr	Trp	Phe	Ile	Ser	Trp	Ser	Pro	Cys	Phe	Ser	Trp	Gly
		275					280					285			
Cys	Ala	Gly	Glu	Val	Arg	Ala	Phe	Leu	Gln	Glu	Asn	Thr	His	Val	Arg
	290					295					300				
Leu	Arg	Ile	Phe	Ala	Ala	Arg	Ile	Tyr	Asp	Tyr	Asp	Pro	Leu	Tyr	Lys
305					310					315				320	
Glu	Ala	Leu	Gln	Met	Leu	Arg	Asp	Ala	Gly	Ala	Gln	Val	Ser	Ile	Met
			325						330					335	
Thr	Tyr	Asp	Glu	Phe	Glu	Tyr	Cys	Trp	Asp	Thr	Phe	Val	Tyr	Arg	Gln
		340						345				350			
Gly	Cys	Pro	Phe	Gln	Pro	Trp	Asp	Gly	Leu	Glu	Glu	His	Ser	Gln	Ala

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355 360 365
 Leu Ser Gly Arg Leu Arg Ala Ile Leu Gln Asn Gln Gly Asn
 370 375 380

<210> 9
 <211> 1536
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequences; note =
 synthetic construct

<400> 9
 acagagcttc aaaaaaagag cgggacaggg acaagcgtat ctaagaggct gaacatgaat 60
 ccacagatca gaaatccgat ggagcggatg tatcgagaca cattctacga caactttgaa 120
 aacgaaccca tcctctatgg tcggagctac acttggctgt gctatgaagt gaaaataaag 180
 aggggccgct caaatctcct ttgggacaca ggggtctttc gaggccaggt gtattttcaag 240
 cctcagtacc acgcagaaat gtgcttcctc tcttggttct gtggcaacca gctgcctgct 300
 tacaagtgtt tccagatcac ctggtttgta tcttggaccc cctgcccgga ctgtgtggcg 360
 aagctggccg aattcctgtc tgagcacccc aatgtcacc tgaccatctc tgccgcccg 420
 ctctactact actgggaaa agattaccga agggcgctct gcaggctgag tcaggcagga 480
 gcccgcgtga cgatcatgga ctatgaagaa tttgcatact gctgggaaaa ctttgtgtac 540
 aatgaaggtc agcaattcat gccttggtag aaattcgatg aaaattatgc attcctgcac 600
 cgcacgctaa aggagattct cagatacctg atggatccag acacattcac tttcaacttt 660
 aataatgacc ctttgggtcct tcgacggcgc cagacctact tgtgctatga ggtggagcgc 720
 ctggacaatg gcacctgggt cctgatggac cagcacatgg gcttttctatg caacgaggct 780
 aagaatcttc tctgtggctt ttacggccgc catgcccggagc tgcgcttctt ggacctggtt 840
 ccttcttttg agttggaccc ggcccagatc tacagggtca cttggttcat ctcctggagc 900
 ccttgcttct cctggggctg tgccggggaa gtgcgtgcgt tccttcagga gaacacacac 960
 gtgagactgc gcatcttcgc tgcccgcatc tatgattacg acccctata taaggaggcg 1020
 ctgcaaatgc tgcgggatgc tggggcccaa gtctccatca tgacctacga tgagtttgag 1080
 tactgctggg acacctttgt gtaccgccag ggatgtccct tccagccctg ggatggacta 1140
 gaggagcaca gccaaagcct gagggtggag ctgcgggcca ttctccagaa tcagggaac 1200
 tgaaggatgg gcctcagtct ctaaggaagg cagagacctg ggttgagcag cagaataaaa 1260
 gatcttcttc caagaaatgc aaacagaccg ttcaccacca tctccagctg ctcacagaca 1320
 ccagcaaagc aatgtgctcc tgatcaagta gattttttta aaatcagagt caattaattt 1380
 taattgaaaa tttctcttat gttccaagtg tacaagagta agattatgct caatattccc 1440
 agaatagttt tcaatgtatt aatgaagtga ttaattggct ccatatttag actaataaaa 1500
 cattaagaat cttccataat tgtttccaca aacact 1536

<210> 10
 <211> 190
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequences; note =
 synthetic construct

<400> 10
 Met Asn Pro Gln Ile Arg Asn Pro Met Lys Ala Met Tyr Pro Gly Thr
 1 5 10 15
 Phe Tyr Phe Gln Phe Lys Asn Leu Trp Glu Ala Asn Asp Arg Asn Glu
 20 25 30
 Thr Trp Leu Cys Phe Thr Val Glu Gly Ile Lys Arg Arg Ser Val Val
 35 40 45
 Ser Trp Lys Thr Gly Val Phe Arg Asn Gln Val Asp Ser Glu Thr His
 50 55 60
 Cys His Ala Glu Arg Cys Phe Leu Ser Trp Phe Cys Asp Asp Ile Leu
 65 70 75 80

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Ser Pro Asn Thr Lys Tyr Gln Val Thr Trp Tyr Thr Ser Trp Ser Pro
      85      90      95
Cys Pro Asp Cys Ala Gly Glu Val Ala Glu Phe Leu Ala Arg His Ser
      100     105     110
Asn Val Asn Leu Thr Ile Phe Thr Ala Arg Leu Tyr Tyr Phe Gln Tyr
      115     120     125
Pro Cys Tyr Gln Glu Gly Leu Arg Ser Leu Ser Gln Glu Gly Val Ala
      130     135     140
Val Glu Ile Met Asp Tyr Glu Asp Phe Lys Tyr Cys Trp Glu Asn Phe
      145     150     155     160
Val Tyr Asn Asp Asn Glu Pro Phe Lys Pro Trp Lys Gly Leu Lys Thr
      165     170     175
Asn Phe Arg Leu Leu Lys Arg Arg Leu Arg Glu Ser Leu Gln
      180     185     190

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<210> 11

<211> 1127

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 11

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ttaaagaggg ctgctcaact gcaaggacgc tgtaagcagg aagagaagcc acagcgcttc      60
agaaaagagt gggacagggg caagcatatc taagaggctg aacatgaatc cacagatcag      120
aaacccgatg aaggcaatgt atccaggcac attctacttc caatttaaaa acctatggga      180
agccaacgat cggaacgaaa cttggctgtg cttcacctgt gaaggtataa agcgccgctc      240
agtgtctctc tggaagacgg gcgtcttccg aaaccagggt gattctgaga ccatttgtca      300
tgcagaaagg tgcttcctct cttgggtctg cgacgacata ctgtctccta acacaaagta      360
ccaggtcacc tggtagacat cttggagccc ttgccagac tgtgcagggg aggtggccga      420
gttcctggcc aggcacagca acgtgaatct caccatcttc accgcccgcc tctactactt      480
ccagtatcca tgttaccagg aggggctccg cagcctgagt caggaagggg tgcgtgtgga      540
gatcatggac tatgaagatt ttaaataattg ttgggaaaac tttgtgtaca atgataatga      600
gccattcaag ccttgaaggg gattaaaaac caactttcga cttctgaaaa gaaggctacg      660
ggagagtctc cagtgagggg tctccctggg cctcatggtc tgtctcctct agcctcctgc      720
tcattgctga cgggcctccc ctccaccctg gaccgctct gttctgcct ggtcatcctg      780
agcccctcct ggccctcagg ccattccaca gtgctcccct gcctcaccgc ttcctcctcg      840
ctcttccaga ctcttctctg agaggctcct ttctgcctcc atggctatcc atccaccccc      900
acagaccccc ttcttccagc ctgctgcccc ctaacctggc ttttcccatc tccccagcat      960
aaccaaatct tactaaactc atcctaggct gggcatggtg actcacgcct gtaatcccc      1020
agcaatttgg gaggcaaagg tgggagaatc gcgtgagccc aggagttcca gaccaggctg      1080
ggtcacatga caaagcccca tctctacaaa aaaaaaaaaa aaaaaaa      1127

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<210> 12

<211> 373

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 12

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Met Lys Pro His Phe Arg Asn Thr Val Glu Arg Met Tyr Arg Asp Thr
  1           5           10          15
Phe Ser Tyr Asn Phe Tyr Asn Arg Pro Ile Leu Ser Arg Arg Asn Thr
      20           25           30
Val Trp Leu Cys Tyr Glu Val Lys Thr Lys Gly Pro Ser Arg Pro Arg

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35	40	45	
Leu Asp Ala Lys Ile Phe Arg Gly Gln Val Tyr Ser Gln Pro Glu His			
50	55	60	
His Ala Glu Met Cys Phe Leu Ser Trp Phe Cys Gly Asn Gln Leu Pro			
65	70	75	80
Ala Tyr Lys Cys Phe Gln Ile Thr Trp Phe Val Ser Trp Thr Pro Cys			
85	90	95	
Pro Asp Cys Val Ala Lys Leu Ala Glu Phe Leu Ala Glu His Pro Asn			
100	105	110	
Val Thr Leu Thr Ile Ser Ala Ala Arg Leu Tyr Tyr Tyr Trp Glu Arg			
115	120	125	
Asp Tyr Arg Arg Ala Leu Cys Arg Leu Ser Gln Ala Gly Ala Arg Val			
130	135	140	
Lys Ile Met Asp Asp Glu Glu Phe Ala Tyr Cys Trp Glu Asn Phe Val			
145	150	155	160
Tyr Ser Glu Gly Gln Pro Phe Met Pro Trp Tyr Lys Phe Asp Asp Asn			
165	170	175	
Tyr Ala Phe Leu His Arg Thr Leu Lys Glu Ile Leu Arg Asn Pro Met			
180	185	190	
Glu Ala Met Tyr Pro His Ile Phe Tyr Phe His Phe Lys Asn Leu Arg			
195	200	205	
Lys Ala Tyr Gly Arg Asn Glu Ser Trp Leu Cys Phe Thr Met Glu Val			
210	215	220	
Val Lys His His Ser Pro Val Ser Trp Lys Arg Gly Val Phe Arg Asn			
225	230	235	240
Gln Val Asp Pro Glu Thr His Cys His Ala Glu Arg Cys Phe Leu Ser			
245	250	255	
Trp Phe Cys Asp Asp Ile Leu Ser Pro Asn Thr Asn Tyr Glu Val Thr			
260	265	270	
Trp Tyr Thr Ser Trp Ser Pro Cys Pro Glu Cys Ala Gly Glu Val Ala			
275	280	285	
Glu Phe Leu Ala Arg His Ser Asn Val Asn Leu Thr Ile Phe Thr Ala			
290	295	300	
Arg Leu Tyr Tyr Phe Trp Asp Thr Asp Tyr Gln Glu Gly Leu Arg Ser			
305	310	315	320
Leu Ser Gln Glu Gly Ala Ser Val Glu Ile Met Gly Tyr Lys Asp Phe			
325	330	335	
Lys Tyr Cys Trp Glu Asn Phe Val Tyr Asn Asp Asp Glu Pro Phe Lys			
340	345	350	
Pro Trp Lys Gly Leu Lys Tyr Asn Phe Leu Phe Leu Asp Ser Lys Leu			
355	360	365	
Gln Glu Ile Leu Glu			
370			

<210> 13

<211> 2672

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 13

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ctctgccagg	gggagggccc	cagagaaaac	cagaaagagg	gtgagagact	gaggaagata	120
aagcgtccca	gggcctccta	caccagcgcc	tgagcaggaa	gggggagggg	ccatgactac	180
gaggccctgg	gaggtcactt	tagggagggg	tgctctgaaa	cctggagcct	ggagcagaaa	240
gtgaaaccct	ggtgctccag	acaaagatct	tagtcgggac	tagccggcca	aggatgaagc	300
ctcacttcag	aaacacagtg	gagcgaatgt	atcgagacac	attctcctac	aacttttata	360
atagacccat	cctttctcgt	cggaataaccg	tctggctgtg	ctacgaagtg	aaaacaaagg	420

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gtccctcaag	gccccgtttg	gacgcaaaga	tcttttcgagg	ccagggtgtat	tcccagcctg	480
agcaccacgc	agaaatgtgc	ttcctctctt	ggttctgtgg	caaccagctg	cctgcttaca	540
agtgtttcca	gatcacctgg	tttgtatcct	ggacccccctg	cccggactgt	gtggcggaagc	600
tggccgaatt	cctggctgag	cacccaatg	tcacctgac	catctccgcc	gcccgcctct	660
actactactg	ggaaagagat	taccgaagg	cgctctgcag	gctgagtcag	gcaggggccc	720
gcgtgaagat	tatggacgat	gaagaatttg	catactgctg	ggaaaacttt	gtgtacagt	780
aaggctcagc	attcatgcct	tggatcaaat	tcgatgacaa	ttatgcattc	ctgcaccgca	840
cgctaaagga	gattctcaga	aacccgatgg	aggcaatgta	tccacacata	ttctacttcc	900
acttttaaaaa	cctacgcaaa	gcctatggtc	ggaacgaaag	ctggctgtgc	ttcaccatgg	960
aagttgtaaa	gcaccactca	cctgtctcct	ggaagagggg	cgtcttccga	aaccaggtgg	1020
atcctgagac	ccattgtcat	gcagaaagg	gcttctctct	ttggttctgt	gacgacatac	1080
tgtctcctaa	cacaaactac	gaggtcacct	ggtacacatc	ttggagccct	tgcccagagt	1140
gtgcagggga	ggtggccgag	ttcctggcca	ggcacagcaa	cgtgaatctc	accatcttca	1200
ccgcccgcct	ctactacttc	tgggatacag	attaccagga	ggggctccgc	agcctgagtc	1260
aggaaggggc	ctccgtggag	atcatgggct	acaaagattt	taaatattgt	tgggaaaact	1320
ttgtgtacaa	tgatgatgag	ccattcaagc	cttggaaagg	actaaaatac	aactttctat	1380
tcttgagacg	caagctgcag	gagattctcg	agtgaagggg	ctccccgggc	ctcatgggtc	1440
gtctcctcta	gcctcctgct	catgttgtgc	aggcctcccc	tccatcctgg	accagctgtg	1500
cttttgccctg	gtcatcctga	gccccctcctg	gcctcagggc	cattccatag	tgctccccctg	1560
cctcaccacc	tcctctccgc	tctcccaggc	tcttctgca	gaggcctctt	tctgctcca	1620
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ctgtcaccca	gactggagtg	caatggcttg	atcttggtct	actgcaaact	ctgcctacca	1800
ggttcaagcg	attctcctgc	ctccgcctcc	cgagtagctg	gaattacaga	cgctgccac	1860
cacgcacagc	taactttttt	tttttttgta	tttttagtag	tgactggggt	tcaccatgtt	1920
ggccaggctg	gtcttgaact	cctgacctca	ggtgatccgc	ctatctcagc	ctcccaaagt	1980
gctgggatta	caggcgtgag	ccactggccc	ggcggcaca	ccaaatctta	ttaaactcac	2040
cctaggtctg	ccgcggtgac	tcatgcctat	aatccccag	caatttgga	ggcagaggtg	2100
agagaatcgc	ttgagcccag	gaattcgaga	ccagcctggg	ccacatgaca	aagccccatc	2160
tctacaaaaa	aattacaaaa	aaaaaaaaaa	caggtgtggt	ggcatgcacc	tgtagtttaa	2220
gctgcttgga	aggatgaagt	gggaggattg	cttgagccgg	ggaggtggag	gctgcagtga	2280
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aatcaataaa	taaactcaac	cgaaatgggt	atgaaagttg	aaatgggtat	gtaagttgaa	2400
aaccagaagt	tttgagaaac	atcctttgtt	aactttcatc	ctacaaattg	ggtcattcat	2460
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cattgctccc	aaaatggact	tctctgcaag	cctgactcct	gaaactgtgc	attgtaccct	2580
gaaaccagct	ttatccatag	cttctgcaat	aaatggctgt	aagtcttgga	aaaaaaaaaa	2640
aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aa			2672

<210> 14

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 14

Pro Pro Leu Pro

1

<210> 15

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

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<400> 15
acagagcttc aaaaaaagag cgggacaggg acaagcgtat ctaagaggct gaacatgaat 60
ccacaga 67

<210> 16
<211> 97
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 16
cctggggctg tgccggggaa gtgcgtgcgt tccttcagga gaacacacac gtgagactgc 60
gcattctcgc tgcccgcatc tatgattacg accccct 97

<210> 17
<211> 194
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 17
ttaaagaggg ctgctcaact gcaaggacgc tgtaagcagg aagagaagcc acagcgcttc 60
agaaaagagt gggacagga caagcatatc taagaggctg aacatgaatc cacagatcag 120
aaacccgatg aaggcaatgt atccaggcac attctacttc caatttaaaa acctatggga 180
agccaacgat cgga 194

<210> 18
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 18
ttccctttgc aattgccttg ggtcctgccg cacagagcgg cctgtcttta tcagagggtcc 60

<210> 19
<211> 397
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 19
aatgatgatg agccattcaa gccttggaag ggactaaaat acaactttct attcctggac 60
agcaagctgc aggagattct cgagtgaagg gtctccccgg gcctcatggg ctgtctcctc 120
tagcctcctg ctcattgtgt gcaggcctcc cctccatcct ggaccagctg tgcttttgcc 180
tggtcatcct gagccctcc tggcctcagg gccattccat agtgctcccc tgccacacca 240
cctcctctcc gctctccag gctcttctg cagaggcctc tttctgcctc catggctatc 300
catccacca ccaagacct gttccctgag cctgcatgcc cctaacctgc cttttcccat 360
ctccccagca taacctata tttttttttt ttttttg 397

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<210> 20
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequences; note =
 synthetic construct

<400> 20
 atactgctta aagtcgtgac aacc

24

<210> 21
 <211> 15
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequences; note =
 synthetic construct

<400> 21
 cacggtggta cttct

15

<210> 22
 <211> 142
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequences; note =
 synthetic construct

<400> 22
 Met Lys Val Gly Gly Ile Glu Asp Arg Gln Leu Glu Ala Leu Lys Arg
 1 5 10 15
 Ala Ala Leu Lys Ala Cys Glu Leu Ser Tyr Ser Pro Tyr Ser His Phe
 20 25 30
 Arg Val Gly Cys Ser Ile Leu Thr Asn Asn Asp Val Ile Phe Thr Gly
 35 40 45
 Ala Asn Val Glu Asn Ala Ser Tyr Ser Asn Cys Ile Cys Ala Glu Arg
 50 55 60
 Ser Ala Met Ile Gln Val Leu Met Ala Gly His Arg Ser Gly Trp Lys
 65 70 75 80
 Cys Met Val Ile Cys Gly Asp Ser Glu Asp Gln Cys Val Ser Pro Cys
 85 90 95
 Gly Val Cys Arg Gln Phe Ile Asn Glu Phe Val Val Lys Asp Phe Pro
 100 105 110
 Ile Val Met Leu Asn Ser Thr Gly Ser Arg Ser Lys Val Met Thr Met
 115 120 125
 Gly Glu Leu Leu Pro Met Ala Phe Gly Pro Ser His Leu Asn
 130 135 140

<210> 23
 <211> 130
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequences; note =

12/24

synthetic construct

<400> 23

```

Met Asn Arg Gln Glu Leu Ile Thr Glu Ala Leu Lys Ala Arg Asp Met
 1           5           10           15
Ala Tyr Ala Pro Tyr Ser Lys Phe Gln Val Gly Ala Ala Leu Leu Thr
           20           25           30
Lys Asp Gly Lys Val Tyr Arg Gly Asn Ile Glu Asn Ala Ala Tyr Ser
           35           40           45
Met Cys Asn Cys Ala Glu Arg Thr Ala Leu Phe Lys Ala Val Ser Glu
           50           55           60
Gly Asp Thr Glu Phe Gln Met Leu Ala Val Ala Ala Asp Thr Pro Gly
65           70           75           80
Pro Val Ser Pro Cys Gly Ala Cys Arg Gln Val Ile Ser Glu Leu Cys
           85           90           95
Thr Lys Asp Val Ile Val Val Leu Thr Asn Leu Gln Gly Gln Ile Lys
           100          105          110
Glu Met Thr Val Glu Glu Leu Leu Pro Gly Ala Phe Ser Ser Glu Asp
           115          120          125
Leu His
           130

```

<210> 24

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 24

```

Glu Asp Ala Leu Ala Phe Ala Leu Leu Pro Leu Ala Ala Ala Cys Ala
 1           5           10           15
Arg Thr Pro Leu Ser Asn Phe Asn Val Gly Ala Ile Ala Arg Gly Val
           20           25           30
Ser Gly Thr Trp Tyr Phe Gly Ala Asn Met Glu Phe Ile Gly Ala Thr
           35           40           45
Met Gln Gln Thr Val His Ala Glu Gln Ser Ala Ile Ser His Ala Trp
           50           55           60
Leu Ser Gly Glu Lys Ala Leu Ala Ala Ile Thr Val Asn Tyr Thr Pro
65           70           75           80
Cys Gly His Cys Arg Gln Phe Met Asn Glu Leu Asn Ser Gly Leu Asp
           85           90           95
Leu Arg Ile His Leu Pro Gly Arg Glu Ala His Ala Leu Arg Asp Tyr
           100          105          110
Leu Pro Asp Ala Phe Gly Pro Lys Asp Leu Glu
           115          120

```

<210> 25

<211> 135

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 25

```

Met Thr Ser Glu Lys Gly Pro Ser Thr Gly Asp Pro Thr Leu Arg Arg
 1           5           10           15

```

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```

Arg Ile Glu Pro Trp Glu Phe Asp Val Phe Tyr Asp Pro Arg Glu Leu
      20      25      30
Arg Lys Glu Ala Cys Leu Leu Tyr Glu Ile Lys Trp Gly Met Ser Arg
      35      40      45
Lys Ile Trp Arg Ser Ser Gly Lys Asn Thr Thr Asn His Val Glu Val
      50      55      60
Asn Phe Ile Lys Lys Phe Thr Ser Glu Arg Asp Phe His Pro Ser Ile
      65      70      75      80
Ser Cys Ser Ile Thr Trp Phe Leu Ser Trp Ser Pro Cys Trp Glu Cys
      85      90      95
Ser Gln Ala Ile Arg Glu Phe Leu Ser Arg His Pro Gly Val Thr Leu
      100      105      110
Val Ile Tyr Val Ala Arg Leu Phe Trp His Met Asp Gln Gln Asn Arg
      115      120      125
Gln Gly Leu Arg Asp Leu Val
      130      135

```

<210> 26

<211> 130

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 26

```

Met Asp Ser Leu Leu Met Asn Arg Arg Lys Phe Leu Tyr Gln Phe Lys
1      5      10      15
Asn Val Arg Trp Ala Lys Gly Arg Arg Glu Thr Tyr Leu Cys Tyr Val
      20      25      30
Val Lys Arg Arg Asp Ser Ala Thr Ser Phe Ser Leu Asp Phe Gly Tyr
      35      40      45
Leu Arg Asn Lys Asn Gly Cys His Val Glu Leu Leu Phe Leu Arg Tyr
      50      55      60
Ile Ser Asp Trp Asp Leu Asp Pro Gly Arg Cys Tyr Arg Val Thr Trp
      65      70      75      80
Phe Thr Ser Trp Ser Pro Cys Tyr Asp Cys Ala Arg His Val Ala Asp
      85      90      95
Phe Leu Arg Gly Asn Pro Asn Leu Ser Leu Arg Ile Phe Thr Ala Arg
      100      105      110
Leu Tyr Phe Cys Glu Asp Arg Lys Ala Glu Pro Glu Gly Leu Arg Arg
      115      120      125
Leu His
      130

```

<210> 27

<211> 138

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 27

```

Met Lys Pro His Phe Arg Asn Thr Val Glu Arg Met Tyr Arg Asp Thr
1      5      10      15
Phe Ser Tyr Asn Phe Tyr Asn Arg Pro Ile Leu Ser Arg Arg Asn Thr
      20      25      30

```

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```

Val Trp Leu Cys Tyr Glu Val Lys Tyr Lys Gly Pro Ser Arg Pro Pro
   35           40           45
Leu Asp Ala Lys Ile Phe Arg Gly Gln Val Tyr Ser Glu Leu Lys His
   50           55           60
Pro Glu Met Arg Phe Glu His Trp Phe Ser Lys Trp Arg Lys Leu His
  65           70           75           80
Arg Asp Gln Glu Tyr Glu Val Thr Trp Tyr Ile Ser Trp Ser Pro Cys
           85           90           95
Thr Lys Cys Thr Arg Asp Met Ala Thr Phe Leu Ala Glu Asp Pro Lys
           100          105          110
Val Thr Leu Thr Ile Phe Val Ala Arg Leu Tyr Tyr Phe Trp Asp Pro
          115          120          125
Asp Tyr Gln Glu Ala Leu Arg Ser Leu Cys
  130           135

```

<210> 28

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 28

```

Glu Pro Trp Val Arg Gly Arg His Glu Thr Tyr Leu Cys Tyr Glu Val
  1           5           10           15
Glu Arg Met His Asn Asp Thr Trp Val Leu Leu Asn Gln Arg Arg Gly
          20          25          30
Phe Leu Cys Asn Gln Ala Pro His Lys His Gly Phe Leu Glu Gly Arg
          35          40          45
His Ala Glu Leu Cys Phe Leu Asp Val Ile Pro Phe Trp Lys Leu Asp
          50          55          60
Leu Asp Gln Asp Tyr Arg Val Thr Cys Phe Thr Ser Trp Ser Pro Cys
  65           70           75           80
Phe Ser Cys Ala Gln Glu Met Ala Lys Phe Ile Ser Lys Asn Lys His
          85          90          95
Val Ser Leu Cys Ile Phe Thr Ala Arg Ile Tyr Asp Asp Gln Gly Arg
          100         105         110
Cys Gln Glu Gly Leu Arg Thr Leu Ala
          115         120

```

<210> 29

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 29

```

Ile Lys Pro Leu Leu Met Asp Glu Gln Asp His Gly Tyr Ala Leu Thr
  1           5           10           15
Gly

```

<210> 30

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 30

Asn Ser Gly Val Thr Ile Gln Ile

1

5

<210> 31

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 31

Arg Ala Gly Val Gln Ile Ala Ile

1

5

<210> 32

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 32

Lys Arg Asp Gly Pro Arg Ala Thr Met Lys Ile

1

5

10

<210> 33

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 33

Glu Ala Gly Ala Lys Ile Ser Ile

1

5

<210> 34

<211> 142

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 34

Met Lys Val Gly Gly Ile Glu Asp Arg Gln Leu Glu Ala Leu Lys Arg

1

5

10

15

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```

Ala Ala Leu Lys Ala Cys Glu Leu Ser Tyr Ser Pro Tyr Ser His Phe
      20      25      30
Arg Val Gly Cys Ser Ile Leu Thr Asn Asn Asp Val Ile Phe Thr Gly
      35      40      45
Ala Asn Val Glu Asn Ala Ser Tyr Ser Asn Cys Ile Cys Ala Glu Arg
      50      55      60
Ser Ala Met Ile Gln Val Leu Met Ala Gly His Arg Ser Gly Trp Lys
      65      70      75      80
Cys Met Val Ile Cys Gly Asp Ser Glu Asp Gln Cys Val Ser Pro Cys
      85      90      95
Gly Val Cys Arg Gln Phe Ile Asn Glu Phe Val Val Lys Asp Phe Pro
      100      105      110
Ile Val Met Leu Asn Ser Thr Gly Ser Arg Ser Lys Val Met Thr Met
      115      120      125
Gly Glu Leu Leu Pro Met Ala Phe Gly Pro Ser His Leu Asn
      130      135      140

```

<210> 35

<211> 131

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =

synthetic construct

<400> 35

```

Met Asn Arg Gln Glu Leu Ile Thr Glu Ala Leu Lys Ala Arg Asp Met
 1          5          10          15
Ala Tyr Ala Pro Tyr Ser Lys Phe Gln Val Gly Ala Ala Leu Leu Thr
      20      25      30
Lys Asp Gly Lys Val Tyr Arg Gly Cys Asn Ile Glu Asn Ala Ala Tyr
      35      40      45
Ser Met Cys Asn Cys Ala Glu Arg Thr Ala Leu Phe Lys Ala Val Ser
      50      55      60
Glu Gly Asp Thr Glu Phe Gln Met Leu Ala Val Ala Ala Asp Thr Pro
      65      70      75      80
Gly Pro Val Ser Pro Cys Gly Ala Cys Arg Gln Val Ile Ser Glu Leu
      85      90      95
Cys Thr Lys Asp Val Ile Val Val Leu Thr Asn Leu Gln Gly Gln Ile
      100      105      110
Lys Glu Met Thr Val Glu Glu Leu Leu Pro Gly Ala Phe Ser Ser Glu
      115      120      125
Asp Leu His
      130

```

<210> 36

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 36

```

Asp Ala Leu Ser Gln Ala Ala Ile Ala Ala Ala Asn Arg Ser His Met
 1          5          10          15
Pro Tyr Ser Lys Ser Pro Ser Gly Val Ala Leu Glu Cys Lys Asp Gly
      20      25      30

```

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```

Arg Ile Phe Ser Gly Ser Tyr Ala Glu Asn Ala Ala Phe Asn Pro Thr
   35           40           45
Leu Pro Pro Leu Gln Gly Ala Leu Ile Leu Leu Asn Leu Lys Gly Tyr
   50           55           60
Asp Tyr Pro Asp Ile Gln Arg Ala Val Leu Ala Glu Lys Ala Asp Ala
  65           70           75           80
Pro Leu Ile Gln Trp Asp Ala Thr Ser Ala Thr Leu Lys Ala Leu Gly
           85           90           95
Cys His Ser Ile Asp Arg Val Leu Leu Ala
           100           105

```

<210> 37

<211> 135

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 37

```

Met Thr Ser Glu Lys Gly Pro Ser Thr Gly Asp Pro Thr Leu Arg Arg
  1           5           10           15
Arg Ile Glu Pro Trp Glu Phe Asp Val Phe Tyr Asp Pro Arg Glu Leu
           20           25           30
Arg Lys Glu Ala Cys Leu Leu Tyr Glu Ile Lys Trp Gly Met Ser Arg
           35           40           45
Lys Ile Trp Arg Ser Ser Gly Lys Asn Thr Thr Asn His Val Glu Val
           50           55           60
Asn Phe Ile Lys Lys Phe Thr Ser Glu Arg Asp Phe His Pro Ser Ile
  65           70           75           80
Ser Cys Ser Ile Thr Trp Phe Leu Ser Trp Ser Pro Cys Trp Glu Cys
           85           90           95
Ser Gln Ala Ile Arg Ser Phe Leu Ser Arg His Pro Gly Val Ile Leu
           100           105           110
Val Ile Tyr Val Ala Arg Leu Phe Trp His Asn Asp Gln Gln Asn Arg
           115           120           125
Gln Gly Leu Arg Asp Leu Val
           130           135

```

<210> 38

<211> 92

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 38

```

Met Arg Ala Ser Glu Tyr Tyr His Cys Trp Arg Asn Phe Val Asn Tyr
  1           5           10           15
Pro Pro Gly Asp Glu Ala His Trp Pro Gln Tyr Pro Pro Leu Trp Met
           20           25           30
Met Leu Tyr Ala Leu Glu Leu His Cys Ile Ile Leu Ser Leu Pro Pro
           35           40           45
Cys Leu Lys Ile Ser Arg Arg Trp Gln Asn His Leu Thr Phe Phe Glu
           50           55           60
Leu His Leu Gln Asn Cys His Tyr Gln Thr Ile Pro Pro His Ile Leu
  65           70           75           80
Leu Ala Thr Leu Ile His Pro Ser Val Ala Trp Arg

```

85

90

<210> 39
 <211> 60
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequences; note =
 synthetic construct

<400> 39
 Met Thr Phe Lys Asp Tyr Phe Tyr Cys Trp Asn Thr Phe Val Glu Asn
 1 5 10 15
 His Glu Arg Thr Phe Lys Ala Trp Glu Gly Leu His Glu Asn Ser Val
 20 25 30
 Arg Leu Thr Arg Gln Leu Arg Arg Ile Leu Leu Pro Leu Tyr Glu Val
 35 40 45
 Asp Asp Leu Arg Asp Ala Phe Arg Thr Leu Gly Leu
 50 55 60

<210> 40
 <211> 57
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequences; note =
 synthetic construct

<400> 40
 Met Asn Tyr Asp Glu Phe Gln His Cys Trp Ser Lys Phe Val Tyr Ser
 1 5 10 15
 Gln Arg Glu Leu Phe Glu Pro Trp Asn Asn Leu Pro Lys Tyr Tyr Ile
 20 25 30
 Leu Leu His Ile Met Leu Gly Glu Ile Leu Arg His Ser Met Asp Pro
 35 40 45
 Pro Thr Phe Thr Phe Asn Phe Asn Asn
 50 55

<210> 41
 <211> 48
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequences; note =
 synthetic construct

<400> 41
 Met Thr Tyr Ser Glu Phe Lys His Cys Trp Asp Thr Phe Val Asp His
 1 5 10 15
 Gln Gly Cys Pro Phe Gln Pro Trp Asp Gly Leu Asp Glu His Ser Gln
 20 25 30
 Asp Leu Ser Gly Arg Leu Arg Ala Ile Leu Gln Asn Gln Gly Glu Asn
 35 40 45

<210> 42
 <211> 40
 <212> PRT
 <213> Artificial Sequence

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<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<221> VARIANT

<222> (12)...(27)

<223> Xaa can be any amino acid

<400> 42

Thr	Asn	His	Val	Glu	Val	Asn	Phe	Ile	Lys	Lys	Xaa	Xaa	Xaa	Xaa	Xaa
1				5				10					15		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Trp	Phe	Leu	Ser	Trp
			20					25					30		
Ser	Pro	Cys	Trp	Glu	Cys	Ser	Gln								
		35					40								

<210> 43

<211> 39

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<221> VARIANT

<222> (12)...(26)

<223> Xaa can be any amino acid

<400> 43

Gly	Cys	His	Val	Glu	Leu	Leu	Phe	Leu	Arg	Tyr	Xaa	Xaa	Xaa	Xaa	Xaa
1				5				10					15		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Trp	Phe	Thr	Ser	Trp	Ser
			20					25					30		
Pro	Cys	Tyr	Asp	Cys	Ala	Arg									
			35												

<210> 44

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<221> VARIANT

<222> (12)...(27)

<223> Xaa can be any amino acid

<400> 44

Lys	Tyr	His	Pro	Glu	Met	Arg	Phe	Phe	His	Trp	Xaa	Xaa	Xaa	Xaa	Xaa
1				5				10					15		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Trp	Tyr	Ile	Ser	Trp
			20					25					30		
Ser	Pro	Cys	Thr	Lys	Cys	Thr	Arg								
		35					40								

<210> 45

<211> 39

<212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
 synthetic construct

<221> VARIANT

<222> (12)...(26)

<223> Xaa can be any amino acid

<400> 45

Gly	Arg	His	Ala	Glu	Leu	Cys	Phe	Leu	Asp	Val	Xaa	Xaa	Xaa	Xaa	Xaa
1				5				10					15		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Phe	Thr	Ser	Trp	Ser
				20				25					30		
Pro	Cys	Phe	Ser	Cys	Ala	Gln									
				35											

<210> 46

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
 synthetic construct

<221> VARIANT

<222> (12)...(22)

<223> Xaa can be any amino acid

<400> 46

Thr	Val	His	Ala	Glu	Gln	Ser	Ala	Ile	Ser	His	Xaa	Xaa	Xaa	Xaa	Xaa
1				5				10					15		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Ile	Thr	Val	Asn	Tyr	Thr	Pro	Cys	Gly	His
				20				25					30		
Cys	Arg	Gln													
				35											

<210> 47

<211> 43

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
 synthetic construct

<221> VARIANT

<222> (12)...(30)

<223> Xaa can be any amino acid

<400> 47

Cys	Ile	Cys	Ala	Glu	Arg	Ser	Ala	Met	Ile	Gln	Xaa	Xaa	Xaa	Xaa	Xaa
1				5				10					15		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Glu	Asp
				20				25					30		
Gln	Cys	Val	Ser	Pro	Cys	Gly	Val	Cys	Arg	Gln					
				35				40							

<210> 48
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 48
Thr Asn His Val Glu Val Asn Phe Ile Lys Lys
1 5 10

<210> 49
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 49
Trp Phe Leu Ser Trp Ser Pro Cys Trp Glu Cys Ser Gln
1 5 10

<210> 50
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 50
Gly Cys His Val Glu Leu Leu Phe Leu Arg Tyr
1 5 10

<210> 51
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 51
Trp Phe Thr Ser Trp Ser Pro Cys Tyr Asp Cys Ala Arg
1 5 10

<210> 52
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =

synthetic construct

<400> 52

Ala Ala His Ala Glu Glu Ala Phe Phe Asn Thr
1 5 10

<210> 53

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 53

Trp Tyr Val Ser Ser Ser Pro Cys Ala Ala Cys Ala Asp
1 5 10

<210> 54

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 54

His Cys His Ala Glu Arg Cys Phe Leu Ser Trp
1 5 10

<210> 55

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 55

Trp Tyr Thr Ser Trp Ser Pro Cys Pro Asp Cys Ala Gly
1 5 10

<210> 56

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 56

Gly Arg His Ala Glu Leu Cys Phe Leu Asp Val
1 5 10

<210> 57

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 57

Cys	Phe	Thr	Ser	Trp	Ser	Pro	Cys	Phe	Ser	Cys	Ala	Gln
1				5						10		

<210> 58

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 58

Lys	Tyr	His	Pro	Glu	Met	Arg	Phe	Phe	His	Trp
1				5					10	

<210> 59

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 59

Trp	Tyr	Ile	Ser	Trp	Ser	Pro	Cys	Thr	Lys	Cys	Thr	Arg
1				5					10			

<210> 60

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 60

Thr	Val	His	Ala	Glu	Gln	Ser	Ala	Ile	Ser	His
1				5					10	

<210> 61

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 61

Ile	Thr	Val	Asn	Tyr	Thr	Pro	Cys	Gly	His	Cys	Arg	Gln
1				5					10			

<210> 62
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 62
Val Cys His Ala Glu Leu Asn Ala Ile Met Asn
1 5 10

<210> 63
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 63
Met Tyr Val Ala Leu Phe Pro Cys Asn Glu Cys Ala Lys
1 5 10